**Justus-Liebig-Universität Gießen**

**Fachbereich Agrarwissenschaften, Ökotrophologie und Umweltmanagement**

Institut für Tierzucht und Haustiergenetik

# Estimation of genetic parameters, phenotype and genomic predictions for novel functional traits in the special context of high-throughput cow genotyping and challenging environmental descriptors

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**Dr. Yin, Tong**

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**List of Abbreviations**

| Abbreviation | Explanation |
|---|---|
| BACT | Basic activity |
| BLUP | Best linear unbiased prediction |
| BW | Body weight |
| BW23 | Body weight recorded at 2 to 3 months of age |
| BW1314 | Body weight recorded at 13 to 14 months of age |
| CH4 | Methane emission |
| DIM | Days in milk |
| DSN | Black and white dual-purpose cattle |
| EBV | Estimated breeding value |
| FEED | Feeding |
| G×E | Genotype by environment interaction |
| GBV | Genomic breeding value |
| GHG | Greenhouse gas |
| GWAS | Genome-wide association study |
| HACT | High activity |
| LD | Linkage disequilibrium |
| LPL | Length of productive life |
| NACT | Resting / non-active |
| PCA | Principal component analysis |
| QTL | Quantitative trait loci |
| $r_{am}$ | Genetic correlations between direct and maternal genetic effects |
| RFLP | Restriction fragment length polymorphism |
| RRM | Random regression model |
| RUM | Ruminating |
| SCS | Somatic cell score |
| SD | Standard deviation |
| SE | Standard error |
| SNP | Single nucleotide polymorphism |
| THI | Temperature humidity index |
| WEL_IC | Welfare index class |
| WEL_IP | Welfare index point |

# 1. General introduction

The main aims of this thesis are 1) to estimate genetic parameters for novel functional traits in dairy cattle considering a broad pattern of environmental descriptors and 2) to evaluate accuracies of estimated breeding values (**EBV**) using pedigree and genomic based approaches. Therefore, this chapter gives an overview for novel functional traits in dairy cattle, provides a short history of genetic markers as applied in animal breeding, addresses statistical approaches in the context of genomic applications (especially from the background of training sets in dairy cattle populations), and introduces important environmental descriptors.

## 1.1. Functional traits

In the dairy cattle sector, the term "functional traits" comprises characteristics of cattle that affect economic efficiency by input cost reductions (Groen et al., 1997). Basically, the functionality for dairy cattle can be classified into five major groups, i.e., health, fertility, calving ease, efficiency and milkability, and every group comprises several novel traits (Groen et al., 1997). Nowadays, in contrast to production traits including milk yield and milk compositions, functional traits are gaining importance in overall dairy cattle breeding goals. For example, health traits (mastitis, metabolic diseases, feet and leg diseases and reproduction disorders) have been accumulatively recorded and genetically evaluated during the past decades in Scandinavia. Live body weight is a very important functional trait in selection indices in some countries with pasture-based farming systems (Pryce and Harris, 2006). In Germany, longevity, 13 direct health traits (mastitis resistance, claw disorders, fertility diseases, and metabolic disorders from the overall categories) and calf/heifer fitness traits are included into the routine national genetic evaluation for Holstein since 2018. Antagonistic genetic associations between production and functional traits encourage direct consideration of functional traits in breeding goals, because solely selection on productivity implies deteriorations of health, fertility, efficiency, and fitness performances. Moreover, the stagnation of milk prices constraints the profit in dairy cattle farming. In order to maintain a certain level of net revenue, it is imperative to reduce involuntary cow cullings to decrease the costs for disease treatments, to increase feed efficiency and to preserve cow fitness. From the perspective of the society, breeding schemes aiming at a balanced improvement of production and functional traits fulfil the consumer acceptance of dairy products.

### 1.1.1. Heat stress response

Climate change is a topic of increasing importance in dairy cattle production systems, encouraging genetic studies on heat stress resistance, and to consider heat stress in genetic evaluations. Based on meteorological data from more than 4,000 weather stations in European countries, the number of days with extreme temperature and wet-bulb temperature were tripled from 1950 to 2018 (Lorenz et al., 2019). In order to investigate the impact of heat stress on production and functional traits, the temperature humidity index (**THI**), (considering dry bulb temperature and relative humidity (NRC, 1971)) has been used as continuous environmental gradient. Ravagnolo et al. (2000) combined test-day records from 15,012 Holstein cows with climatic information from the closest weather station. They identified a production trait decline for THI ≥ 72. Moreover, Ravagnolo and Misztal (2000) reported an alteration of additive genetic variances for heat stress at THI ~ 72. In consequence, THI 72 (when using the NRC equation for the THI calculation) is generally accepted as heat stress threshold in dairy cattle populations. However, in some studies, detrimental impact has been identified for lower THI. In this regard, a decrease in semen productivity for German Holstein Friesian sires (Al-Kanaan et al., 2015) and in female fertility for German Holstein cows (Brügemann et al., 2013) was observed for THI > 60. The threshold for production deteriorations and for detrimental impact on energy indicator traits in high yielding Holstein cows from large-scale cooperator herds was THI 68 (Gernand et al., 2019). For the health status of lactating cows and considering meteorological data from on-farm measurements, identified heat stress thresholds were specific for different health disorders (Gernand et al., 2019). Hence, heat stress thresholds varied among traits and across countries, production systems and populations.

Innovative statistical modelling approaches allow simultaneous consideration of continuous time scales and of environmental gradients. In this regard, variance components for additive genetic and permanent environmental effects estimated via random regression models (**RRM**) differed along the grid pattern combining days in milk (**DIM**) and THI (e.g., Brügemann et al., 2011; Bohlouli et al., 2019). Brügemann et al. (2011) applied a RRM with a pedigree-based relationship matrix. As an example, the heritability for daily protein yield was 0.16 at DIM 5 combined with THI 72, but 0.37 at DIM 305 combined with THI 21. Bohlouli et al. (2019) focused on genomic RRM, and confirmed the heritability increase for milk yield with increasing DIM and decreasing THI. Low genetic correlations (< 0.50) between milk yield recorded at moderate and at extreme THI (Bohlouli et al., 2019) indicate possible genotype by environment interactions (**G×E**) for milk performances when considering “well-being” and

heat stress conditions. In consequence, a heat stress interaction term should be considered in genetic evaluations for production as well as for functional traits. Studies addressing prediction accuracies considering heat stress interactions and genetic parameters for test-day production traits in the context of THI are presented in the **original research papers 1** and **2** of this thesis.

### 1.1.2. Greenhouse gas emissions

Productivity and functionality of dairy cattle react sensible to heat stress. Vice versa, the modern dairy cattle industry is one of the major causes contributing to climate change. The dairy cattle sector generates about 4% of the total global anthropogenic greenhouse gas (**GHG**) emissions (Food and Agriculture Organization of the United Nations., 2010). Methane (**CH4**), the most detrimental GHG from dairy cattle farming, is a by-product of fermentation processes in ruminants, and is 25 times more potent than $CO_2$ to global warming. Consideration of individual CH4 output in breeding approaches might genetically influence feed efficiency indicators, such as dry matter intake (de Haas et al., 2011), residual feed intake (Yan et al., 2010), and gross energy intake (Moraes et al., 2014). Biologically, CH4 emissions from ruminants can be classified into three physiological pathways: 1) CH4 derived from rumen and lower gut which is absorbed into blood and exhaled from the lungs via expiration; 2) CH4 emitted directly from rumen by eructation, and 3) CH4 produced from hindgut and released in flatus and respiration (Ricci et al., 2014; Hammond et al., 2016). Basically, CH4 measurements taken directly from nostrils of ruminants account for most of the enteric CH4 produced in the rumen and hindgut, because a large proportion of CH4 produced in hindgut is also absorbed into blood and emitted through expiration. The proportion of CH4 released via flatus in relation to the total CH4 emission was only 2% (Murray et al., 1976). Therefore, in addition to the expensive but most accurate CH4 measurements in the respiration chamber, technical instruments with focus on CH4 exhaled in normal breath, such as the handheld laser methane detector, quantify enteric CH4 with high accuracy (Chagunda and Yan, 2011). Due to the different recording techniques, CH4 measurements are expressed in several units, such as the CH4 emission rate (in g/d or L/d), the CH4 concentration (in ppm), and CH4 yield (in g CH4/kg dry matter intake). Heritabilities ranged from 0.21 to 0.29 for the CH4 emission rate (Lassen and Løvendahl, 2016; Pinares-Patiño et al., 2013), from 0.05 to 0.14 for the CH4 concentration (Pickering et al., 2015; Paganoni et al., 2017), and from 0.13 to 0.2 for CH4 yield (Pinares-Patiño et al., 2013; Donoghue et al., 2015). In addition to real CH4 emissions recorded from different technical devices, CH4 emissions can be predicted based on correlated indicator traits

and considering specific environmental and feeding conditions. Different equations have been introduced to predict daily CH4. For example, Kirchgessner et al. (1995) reported an equation only including milk yield and metabolic body weight. de Haas et al. (2011) derived CH4 emissions from feed intake and maintenance levels. Alternatively, milk fatty acid composition in milk mid-infrared spectra data was utilized to predict enteric CH4, because of the shared biochemical pathways among CH4, acetate, and butyrate in the rumen (Lassen and Løvendahl, 2016). Using data from Brown Swiss cows kept on low-input farms in mountainous regions of Switzerland, Yin et al. (2015) compared the two prediction equations as introduced before. The same authors stated that the phenotypic and genetic correlations between the two predicted CH4 parameters were larger than 0.8 across lactation. Detailed curves for predicted CH4 emissions and corresponding heritabilities in dependency of DIM, as well as genetic correlations between predicted CH4 and production, fertility and health traits, are presented in the **original research paper 3**.

### 1.1.3. Body weight

Body weight (**BW**) of dairy cattle is a valuable indicator to predict feed efficiency, energy balance and maintenance costs. In consequence, BW has been included in national selection indexes in several countries (Pryce et al., 2015). Genetic correlations of 0.76 and 0.45 were estimated between BW after first calving with dry matter intake and energy balance, respectively (Veerkamp et al., 2000). Recently, Li et al. (2018) reported genetic correlations between BW and feed intake slightly larger than 0.3 across first lactation. Besides the favorable correlations with other breeding goal traits or energy efficiency indicators, moderate to large heritabilities, e.g., 0.45 for weaning weight, 0.75 for calving weight, and 0.35 for weekly averaged live weight (Lassen and Løvendahl, 2016), indicate substantial selection response for BW when implementing direct BW selection strategies. Some large-scale dairy cattle farms in Germany routinely record BW at birth and around the first insemination date, because BW at these time points was closely correlated with test-day production and female fertility traits (Yin and König, 2018). However, due to the variation in the length of energy deficiency periods and the levels of energy deficiency with aging, genetic correlations between BW and productivity altered on the time scale (Veerkamp et al., 2001). Thus, it is imperative to focus on longitudinal BW data as generated in experimental farms or in commercial farms with automatic milking or weighing systems, instead of using single BW measurements from distinct time points. Brotherstone et al. (2007) and Yin and König (2018) estimated heritabilities for longitudinal

BW from birth to first calving via RRM applications. Body weight heritability curves altered with age, but the curve pattern was very similar in both studies. In contrast, Li et al. (2018) estimated quite constant heritabilities for BW by DIM in first parity Holstein cows. Genetic correlations between weekly BW and weekly dry matter intake increased at the beginning of lactation with a "peak estimate" of 0.7 in lactation week 7, but decreased afterwards (Li et al., 2018). The variation in genetic correlation estimates by DIM also suggests longitudinal BW recording. Such aspects, additionally addressing covariance components among BW, productivity, functionality and health traits, are content of the **original research papers 4** and **5**. As a further novelty, **original research paper 6** displays variance components for growth curve parameters from three non-linear functions combined with different genomic kernel matrices.

### 1.1.4. Behavior traits

Schutz and Pajor (2001) examined the role of behavior traits in dairy cattle genetic selection schemes. Their review focused on feeding, reproductive and maternal behavior, temperament and social interactions. Further "natural behavior" categories reflecting biological rhythms and sleep are not under genetic control (Hohenboken, 1986). Basically, subjectively scored temperament traits (scoring done by milking persons) displayed small to moderate heritabilities in the range from 0.09 (Wethal et al., 2020) to 0.22 (Visscher and Goddard, 1995). Milking temperament of dairy cows strongly influences labor time and labor quality in the milking parlor. In consequence, milking temperament is already included into overall breeding goals in many countries. In addition, milking speed, which is moderately correlated with milking temperament (Schutz and Pajor, 2001), is also considered in breeding objectives. Generally, newborn calves are separated from their dams directly after calving. From an animal welfare perspective, the dam should have the possibility to lick its calf, which stimulates the calf to breathe, to suckle, and ultimately contributes to calf vitality. Also, such dam – calf behavior interactions are partly under genetic control (Schutz and Pajor, 2001). As pointed out by Schutz and Pajor (2001), feeding behavior (e.g., the number of daily meals, meal size, chewing time, eating time or drinking time), drinking behavior (e.g., the frequency and amount of water consumption) as well as rumination time play an important role with regard to overall productivity. Aiming on the understanding of the genetic architecture of cattle behavior, and in order to infer correlations between behavior traits and other traits of economic importance, the availability of dense behavior trait pattern is imperative. Hence, automatic or electronic

recording systems should be implemented, e.g., sensor technology. Sensors in combination with location detectors can be applied to determine rumination and eating time with high accuracy. Activities of cows detected via electronic motion sensors plus temperature measurements via heat mount detectors might indicate estrus periods. In consequence, the **original research paper 7** focuses on the estimation of genetic parameters for behavior traits based on ear tag sensor recording, such as rumination time, feeding time, basic active time, high active time and not active time. Furthermore, genetic associations between those behavior traits with production traits are addressed. A further novelty is an across-country approach considering genomic data from multiple breeds, in order to detect genomic regions and potential candidate genes for dairy cow behavior.

### 1.2. Molecular markers in animal breeding

Molecular markers, defined as DNA polymorphisms among species and among individuals within a species (Beuzen et al., 2000), can be classified into different types. Three types are mainly used in animal breeding and genetics, including restriction fragment length polymorphisms (**RFLP**), microsatellites, and single nucleotide polymorphisms (**SNP**). One example for the successful application of RFLPs is the following. In 1990, the bovine leukocyte adhesion deficiency, which is caused due to a single point mutation in the *CD18* gene, became an important autosomal recessive congenital disease, because heterozygous carriers were among the most prominent bulls (Nagahata, 2004). Hence, a polymerase chain reaction-RFLP screening test for the *CD18* mutation was applied, in order to identify carriers and to avoid matings between carriers. Such strategy successfully decreased the prevalence of bovine leukocyte adhesion deficiency (Nagahata, 2004). Hence, RFLP were mostly used to detect genetic defects, i.e., genetic disorders with classic Mendelian inheritance. Microsatellites, which contain two to six nucleotide repeats, are more interesting in quantitative genetic approaches, because they can be used to construct the linkage map for domestic animals (Rohrer et al., 1994). The map provides the framework for identifying genes that contribute to economic and functional traits, and the linkage between markers and quantitative trait loci (**QTL**) can be used in marker assisted selection schemes. For example, using 159 autosomal microsatellites covering approximately two thirds of the bovine genome, Georges et al. (1995) mapped QTL underlying the genetic variation of milk production in an elite Holstein cattle population. A SNP is a substitution of one nucleotide for another, or an insertion or a deletion of one or a few nucleotides in the genome (Beuzen et al., 2000). Basically, microsatellites are

more informative than SNP, because microsatellites contain multi-allelic variants, but SNP mostly represent two alleles. Nevertheless, nowadays, SNP are the commercially used genetic markers for routine animal breeding approaches. The arguments are the following: 1) the large number of valid SNP equally distributed across the genome implies marker locations in neighboring distance to a QTL which directly affects a protein function; 2) the stable inheritance of SNP markers allows efficient long-term selection approaches (Beuzen et al., 2000), and 3) from a technical perspective, high throughput SNP chips can be efficiently created using DNA microarrays (Lipshutz et al., 1999). The size of SNP chips for routine applications increased within a few years from 10k (low-density chip) to 777k (high-density chip). Recently, whole genome sequencing data considering up to 15.8 million SNP and 1.6 million indel variants for Holstein Friesian and Simmental can be used in the context of next generation sequencing (Hayes et al., 2012). However, due to the quite small effective population size and quite high level of linkage disequilibrium (**LD**) in dairy cattle (Goddard and Hayes, 2009), a medium-density 50k SNP chip is sufficient to achieve reliable prediction accuracies in genomic evaluations (Erbe et al., 2012). Therefore, in this thesis, most of the research papers consider 50k or low-density 10k genotypes, but also imputations were applied.

### 1.3. Genome-wide association studies

Genome-wide association studies (**GWAS**) have been applied in animals, plants and humans, aiming on the identification of QTL underlying the traits of interest at a population level. The simplest statistical model to exploit the association between a marker and a trait is single marker regression, implying the regression of phenotypic records for the trait of interest on the number of copies for the respective allele at each locus, one by one. The general statistical model for the single marker regression is:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{W}g + \mathbf{e}$$

where **y** = a vector of phenotypes for the trait of interest; **b** = a vector of fixed effects, e.g., herd, year, calving age, etc.; $g$ = effect of the marker; **e** = vector for random residuals following $N(\mathbf{0}, \sigma_e^2)$; **W** and **X** = incidence matrices allocating the fixed effects (**b**) and the marker effect ($g$) to phenotypic records, respectively. Actually, both **b** and $g$ are treated as fixed effects, but the effects of the marker ($g$) and its incidence matrix (**W**) change, due to the consecutive analyses for the single SNP. Significance of the individual markers can be tested through F test statistics, considering estimates from $\hat{g}$, $\hat{b}$, **y**, **X** and **W** (Hayes and Daetwyler, 2015), or through

likelihood-ratio tests comparing likelihoods of models with and without the marker effect (plus an ongoing test statistic following a $\chi^2$ distribution with 1 degree of freedom under the null hypothesis) (Yang et al., 2011).

However, false positive SNP associations from the simple marker regression model are reported frequently, since it does not take population structure into account (Pritchard et al., 2000). In dairy cattle, an elite bull might produce thousands of daughters, because of artificial insemination and international genetic evaluations as well as similar international selection strategies considering similar breeding goals. Besides, breeds with similar genetic background (e.g., Holstein Friesian and Deutsches Schwarzbuntes Niederungsrind) are included into the same genetic evaluation processes, irrespective of breed or production systems particularities. Phenotypes and genotypes from different breeds and countries can be combined, in order to increase the number of records and power of GWAS. However, in this regard, population stratification in dairy cattle is more prevalent than in human studies. Therefore, a modified single marker regression model with polygenic effects is recommended, since this alternative can remove false positive signals caused by the population structure. In matrix notation, the respective mixed model is:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{W}g + \mathbf{Zu} + \mathbf{e}$$

where **y**, **b**, **g**, **e**, **X** and **W =** notations as defined for the single marker regression model above; **u** = a vector of polygenic effects with a variance-covariance structure of $N$ ($\mathbf{0}$, $\mathbf{K}\sigma_u^2$), where **K** = relationship matrix between animals and $\sigma_u^2$ = polygenic variance; **Z** = incidence matrix allocating animal polygenic effects (**u**) to phenotypic records. The relationship matrix **K** can be the pedigree-based relationship matrix **A**, the genomic relationship matrix **G** (VanRaden, 2008), or other matrices representing similarities between individuals (Schaid, 2010b). Alternatively, the **K** matrix or genotypes of the involved animals can be converted into linearly uncorrelated variables, i.e., into principal components. As implemented in human GWAS, the first principal components which explain a certain amount of variation are included in the single marker regression model and in the mixed model as additional fixed effects, in order to account for population structure (Mahmoud et al., 2018). However, principal components approaches should be conducted with caution, due to ambiguous corrections which are difficult to follow (Daetwyler et al., 2012).

Several factors determine the power of GWAS. First, larger LD between SNP and QTL increased the detection power significantly (Wang and Xu, 2019). The power is maximized

when the SNP is in complete LD with the causative mutation. Increasing the number of SNP contributes to shorter distances between markers and QTL, with positive impact on the detection power. Also, the significant threshold according to the stringent Bonferroni correction depends on the number of tested markers. The second important factor influencing detection power is the proportion of the total phenotypic variance explained by the QTL. For monogenic traits, e.g., polled in cattle, and multifactorial traits with major QTL (e.g. the *DGAT1* gene for milk fat content), GWAS inferred causative mutations with high accuracy (Coppieters et al., 1998). However, in dairy cattle, multiple genes with only minor effects plus environmental effects simultaneously contribute to trait expressions. The third important factor is the sample size. Pritchard and Przeworski (2001) stated that the sample size depends on a factor of $1/r^2$, with $r^2$ being the LD between marker and QTL. Additionally, the detection power was different for loci with a minor allele frequency < 0.1 compared to loci with a minor allele frequency < 0.4 (Ardlie et al., 2002). Therefore, SNP filtering prior to GWAS is recommended. Finally, the detection power depends on the significance threshold. Because of the problem of multiple testing in GWAS (usually testing thousands of markers), an adjusted significant level is necessary to account for false positive associations. In this regard, Bonferroni correction adjusts for the number of markers, in spite of the fact that markers on the same chromosome are not independent. Thus, relaxed significant thresholds, such as a threshold based on a permutation test (Churchill and Doerge, 1994), the false discovery rate (Benjamini and Hochberg, 1995), or a Bonferroni correction considering the number of independent markers (Pausch et al., 2011), are suggested alternatives.

In this thesis, GWAS were conducted to inferring direct and maternal genetic effects on BW at birth and at first insemination (see **original research paper 5**). Additional, one study focused on a multi-breed GWAS and on the annotation of potential candidate genes associated with behavior (e.g., rumination, feeding and activity pattern) in **original research paper 7**.

### 1.4. Similarity matrices in genomic predictions

GWAS approaches identified numerous potential candidate genes and unraveled genetic mechanisms for many traits in dairy cattle. However, most of the quantitative traits are controlled by an infinite number of QTL with infinitely small effects. Hence, statistical models which consider effects of all involved genes, are widely used in genetic evaluations. In this

regard, the simplest statistical model is an animal model (being also the basis for enhanced other models). The animal model is very similar to the GWAS model with polygenic effects:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e}$$

where **y**, **b**, **u**, **e**, **X** and **Z =** as defined for in the GWAS model above. However, the single marker regression coefficient is excluded from the animal model. The expectation of the variables are: E(**y**) = **Xb**; E(**u**) = E(**e**) = 0. Assuming independently distributed residual effects with variance of $\sigma_e^2$, var(**e**) = $\mathbf{I}\sigma_e^2$ = **R**; var(**u**) = $\mathbf{K}\sigma_u^2$ = $\mathbf{G_u}$ and cov(**u**, **e**) = cov(**e**, **u**) = 0. Henderson (1975) developed best linear unbiased prediction (**BLUP**) to solve linear mixed models, allowing the estimation of solutions for fixed effects and breeding values simultaneously with maximized reliability. The mixed model equation for BLUP is:

$$\begin{bmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Z} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z} + \mathbf{G_u}^{-1} \end{bmatrix} \begin{bmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{y} \end{bmatrix}$$

Assuming that **R** and $\mathbf{G_u}$ are non-singular matrices and since **R** = $\mathbf{I}\sigma_e^2$, then the equation can be simplified to:

$$\begin{bmatrix} \mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{Z} \\ \mathbf{Z}'\mathbf{X} & \mathbf{Z}'\mathbf{Z} + \lambda\mathbf{K}^{-1} \end{bmatrix} \begin{bmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{y} \\ \mathbf{Z}'\mathbf{y} \end{bmatrix}$$

where $\lambda = \sigma_e^2/\sigma_u^2$. As mentioned above, the similarity matrix **K** can be constructed according to different approaches. The most traditional approach is to build the matrix based on pedigree information, i.e., the **A** matrix, which reflects the expected additive genetic relationship among individuals. Availability of high-throughput SNP across the whole genome enables calculations of realized relationships, accounting for the Mendelian sampling term. For example, the **G** matrix allows relationship coefficients for full-sibs around 0.5, instead of being exactly equal to 0.5 as defined for the **A** matrix. In consequence, EBVs of non-phenotyped full-sibs could differ, leading to improved prediction accuracies especially for young animals without own performances or without daughter records. The **G** matrix can be constructed according to different formulas. First, VanRaden (2008) proposed a **G** matrix with elements calculated as $\frac{\mathbf{ZZ'}}{\sum 2p_i(1-p_i)}$, where $\mathbf{Z} = \mathbf{M} - \mathbf{P}$. **M** is a matrix containing the genotypes (coded as 0, 1, or 2) of the animals, **P** is a matrix with the $i^{th}$ column equals to two times $p_i$, and $p_i$ is the allele frequency for marker i in the genotyped population. Nowadays, this is the most popular method to create **G**, and defined as default **G** matrix in software packages used for genomic evaluations, such as in the BLUPF90 package (Misztal et al., 2002) and in DMUv6 (Madsen

and Jensen, 2013). Alternatively, the centralized **Z** matrix can be scaled with $\sqrt{2p_i(1-p_i)}$, implying $\frac{\mathbf{ZDZ'}}{m}$, where m is the number of markers used to create the **G** matrix, and **D** is a m-by-m diagonal matrix with $\frac{1}{2p_i(1-p_i)}$ on the diagonal (Amin et al., 2007). Furthermore, Yang et al. (2010) adjusted for the sampling error associated with each SNP. In this regard, they stated that the relationship between two different individuals should be calculated according to Amin et al. (2007), but when focusing on the relationship between the same animal, the formula changed to $1+\frac{1}{m}\sum_{i=1}^{m}\frac{x_{ij}^2-(1+2p_i)x_{ij}+2p_i^2}{2p_i(1-p_i)}$, where $x_{ij}$ = genotype at marker *i* for animal *j*. Legarra et al. (2009) computed a combined **H** matrix by blending **A** and **G** matrices, implying the following inverse of the **H** matrix:

$$\mathbf{H^{-1}} = \mathbf{A^{-1}} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{G^{-1}} - \mathbf{A_{22}^{-1}} \end{bmatrix}$$

where $\mathbf{A_{22}}$ = the submatrix of the pedigree-based relationship matrix for genotyped animals. The regular **G** matrix can be replaced by $\mathbf{G_w} = (1-w)\mathbf{G} + w\mathbf{A_{22}}$, with *w* = the ratio of the residual polygenic variance in relation to the total additive genetic variance (Christensen and Lund, 2010). In practice, the value for *w* is set to a small value in single-step genomic prediction models, such as 0.1 (Gao et al., 2018) and 0.05 (Naderi et al., 2018).

In addition to relationship coefficients calculated based on pedigree or marker information, the variance-covariance structure between individuals can be constructed using all or a subset of markers (**X**) through a nonparametric function g(**X**) (Gianola and van Kaam, 2008). The function g(**X**) is assumed to be a function space $\mathscr{H}_K$, which is generated by a positive definite kernel function $K(\cdot,\cdot)$ under theory of reproducing kernel Hilbert space (RKHS). According to the Representer theorem (Kimeldorf and Wahba, 1971), the solution of g(**X**) = **Kα**, where **K** is a n-by-n kernel matrix (n = no. genotyped animals) and $\boldsymbol{\alpha} = (\alpha_1, \ldots, \alpha_n)$ is a vector of unknown parameters. Element (i, j) in **K** is K($x_j$, $x_k$), which is calculated by the reproducing kernel function based on the genotypes of animal *j* and animal *k*. In a RKHS model with fixed effects and genotypes of animal, the respective equation is:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{K\alpha} + \mathbf{e}$$

where **y**, **b**, **X**, **e** = as defined for the animal model; **α** = a vector of unknown parameters $(\alpha_1, \ldots, \alpha_n)$; **K** = n-by-n kernel similarity matrix. As shown by Liu et al. (2007), **b** and **α** can be estimated by minimizing the residual sum of squares and the penalty for **α**, $\mathbf{J}(\boldsymbol{\beta}, \boldsymbol{\alpha}) =$

$(\boldsymbol{y}-\mathbf{Xb}-\mathbf{K\alpha})'(\boldsymbol{y}-\mathbf{Xb}-\mathbf{K\alpha})+\boldsymbol{\lambda\alpha}'\mathbf{K}$. Differentiating $\mathbf{J}(\mathbf{b},\boldsymbol{\alpha})$ with respect to $\mathbf{b}$ and $\boldsymbol{\alpha}$ leads to $\mathbf{X}'\mathbf{Xb}+\mathbf{X}'\mathbf{K\alpha}-\mathbf{X}'\boldsymbol{y}=\mathbf{0}$ and $\mathbf{K}'\mathbf{Xb}+\mathbf{K}'\mathbf{K\alpha}+\boldsymbol{\lambda}\mathbf{K\alpha}-\mathbf{K}'\boldsymbol{y}=\mathbf{0}$, or in equation form:

$$\begin{bmatrix}\mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{K}\\ \mathbf{K}'\mathbf{X} & \mathbf{K}'\mathbf{K}+\lambda\mathbf{K}\end{bmatrix}\begin{bmatrix}\hat{\mathbf{b}}\\ \hat{\boldsymbol{\alpha}}\end{bmatrix}=\begin{bmatrix}\mathbf{X}'\boldsymbol{y}\\ \mathbf{K}'\boldsymbol{y}\end{bmatrix}$$

then, $\boldsymbol{\alpha}\sim N(\mathbf{0},\mathbf{K}^{-1}\sigma_\alpha^2)$ where $\sigma_\alpha^2$ = variance of $\boldsymbol{\alpha}$ and $\lambda=\sigma_e^2/\sigma_\alpha^2$. Because $\mathbf{K}$ is a symmetric matrix and $\mathbf{K}^{-1}$ exists, the second equation is $\hat{\boldsymbol{\alpha}}=(\mathbf{K}+\boldsymbol{\lambda}\mathbf{I})^{-1}(\mathbf{y}-\mathbf{X}\hat{\mathbf{b}})$. Accordingly, $\hat{\mathbf{g}}=\mathbf{K}\hat{\boldsymbol{\alpha}}=\mathbf{K}(\mathbf{K}+\lambda\mathbf{I})^{-1}(\mathbf{y}-\mathbf{X}\hat{\mathbf{b}})=(\mathbf{I}+\lambda\mathbf{K}^{-1})^{-1}(\mathbf{y}-\mathbf{X}\hat{\mathbf{b}})$, which connects with the linear mixed model (Liu et al., 2007), because the equation form is:

$$\begin{bmatrix}\mathbf{X}'\mathbf{X} & \mathbf{X}'\\ \mathbf{X} & \mathbf{I}+\lambda\mathbf{K}^{-1}\end{bmatrix}\begin{bmatrix}\hat{\mathbf{b}}\\ \hat{\mathbf{g}}\end{bmatrix}=\begin{bmatrix}\mathbf{X}'\boldsymbol{y}\\ \boldsymbol{y}\end{bmatrix}$$

The equation is identical to the normal equation from the linear mixed model:

$$\mathbf{y}=\mathbf{Xb}+\mathbf{g}+\mathbf{e}$$

where $\mathbf{g}$ = a vector of random effects. Therefore, the restricted maximum likelihood algorithm as implemented in standard software packages for mixed models can be used to estimate $\sigma_\alpha^2$ and $\sigma_e^2$, and in further consequence, to estimate the tuning parameter $\lambda$ (Liu et al., 2007; Wang et al., 2015).

Hence, choosing the appropriate kernel function is of great importance in the RKHS regression, since the impact of marker information on traits of interest are expressed only in the kernel matrix **K**, which is produced from the kernel function. Because a new kernel can be created either from a symmetric function that generates a positive definite matrix or from an existing kernel via multiplication with other kernels (Schaid, 2010a), the **K** matrix captures additive genetic as well as nonlinear effects. For example, pairwise **K**×**K** interaction effects among SNP located in 186 unique candidate genes which were associated with birth weight were tested, and 23 gene pairs were significant at 0.001 significance level (Li and Cui, 2012). Schaid (Schaid, 2010b; Schaid, 2010a) reviewed various kernel functions, such as a weight linear kernel (Wu et al., 2011), a polynomial kernel (Zien et al., 2000), a Gaussian kernel (Mallick et al., 2005), and binomial and trinomial kernels (González-Recio et al., 2008). On the basis of all kernels, similarities or dissimilarities between individuals can be measured.

In the **original research paper 1**, predictive abilities from a genomic RRM and considering the **G** matrix, are presented for trait responses in extreme THI classes through simulations. The **original research paper 2** focuses on the impact of heat stress on production traits considering

genotypes of cows and sires as well as the pedigree information, implying single-step genomic predictions. Finally, prediction accuracies for animal models using realized genomic relationship matrices and different kernel matrices are content of the **original research paper 6**.

### 1.5. Cow training sets

In the starting period of genomic selection in cattle, only bulls with highly reliable EBV were genotyped. Hence, breeding organizations mainly focused on improving the male selection pathway. Furthermore, the highly reliable sire EBV based on progeny testing contributed to the development of accurate SNP prediction equations. However, in comparison to the whole population, the number of elite sires with a large number of daughter records is quite small per generation, implying that only a limited number of animals can be added into the training set per year. Additionally, SNP effects and genomic breeding values (**GBV**) estimated based on EBV of intensively pre-selected bulls might not represent the genetic architecture of the production population (Patry and Ducrocq, 2011). Only considering pre-selected sires for genotyping might contribute to biased GBV, especially when applying BLUP methodology. Hence, from such perspective, it is imperative to consider cows for genotyping, and to implement cow training sets. The decrease of genotyping costs was a major impulse for large-scale cow genotyping with a low-/medium-density chip. In several countries, the genotyped cows are gradually included into the training sets, with the aim to achieve large reference populations and an adequate representation of the population. For example, the German "Kuh-L project" (Naderi et al., 2018) focused on genomic selection innovations considering high-throughput genotypes from 20,000 cows. Buch et al. (2012) especially favored cow training sets for low heritability functional traits, which are not considered in official recording schemes. For these traits, Buch et al. (2012) found higher GBV accuracies when deriving SNP effects based on cow phenotypes instead of daughter yield deviations. Consideration of daughter yield deviations from genotyped bulls in genomic prediction ignores the variations within a group of daughters. Therefore, compared to individual cow phenotypes, the information content is reduced when average daughter records per sire are used as pseudo-phenotype. The inclusion of cows into training sets was associated with increased reliabilities of genomic predictions, reduced rate of inbreeding and decreased variance for the selection response in small dairy cattle populations (Thomasen et al., 2014). In small populations, the reliability of EBV for the proven bulls is often restricted due to the small number of animals available for progeny testing,

suggesting to derive SNP effects based on individual cow records. Cow training sets also improved prediction accuracies in large populations. As an example, in a simulation study with 420,000 daughter records, adding genotypes and phenotypes of cows into training sets contributed to an increase of GBV reliabilities for young selection candidates (Plieschke et al., 2016). The authors (Plieschke et al., 2016) also recommended combined cow and bull training sets to avoid biased breeding value estimations caused by intensive pre-selection of young bulls.

Another argument for the implementation of cow training sets are genetic improvements for functional traits, especially when these traits are very difficult or expensive to measure. Methane emission, longitudinal BW and behavior traits are examples in this regard, and addressed via genomic selection based on cow training sets in this thesis. Other examples for successful implementations of cow training sets include health disorders, such as resistance to specific pathogens (Mahmoud et al., 2018), residual feed intake (Pryce et al., 2012), milk fatty acid composition (Gebreyesus et al., 2019) and longevity (Shabalina et al., 2020). In a stochastic simulation study, Plieschke et al. (2018) verified the increase of GBV reliabilities for low heritability novel functional traits when considering genotypes and phenotypes from cows. Detailed benefits from an economic perspective when focusing on cow genotyping are topic of the **original research paper 9** of this thesis. Furthermore, the comprehensive simulation study (**original research paper 1**) and the studies addressing real functional traits, i.e., heat stress in the **original research paper 2**, BW in the **original research paper 5**, growth curve parameters in the **original research paper 6**, and behavior traits in the **original research paper 7**, mainly rely on cow training sets for genomic predictions and genetic parameter estimations.

### 1.6. Environmental descriptors

Because of worldwide utilization of artificial insemination, a great proportion of semen from same bulls is spread over different countries and production systems. However, differences in, e.g., climatic conditions and management characteristics might contribute to possible genotype by environment interactions, indicating different genotype responses with environmental alterations. Both discrete and continuous environmental descriptors have been considered in genetic-statistical modelling approaches, in order to depict environmental variations. Genetic correlations between the same traits recorded in different environments are generally used as indicators for possible G×E, with a genetic correlation threshold of 0.80 (Robertson, 1959).

### 1.6.1. Discrete environmental descriptors

A very basic discrete descriptor is country, because production systems and climatic zones might be extremely different between countries. For example, the genetic correlation between milk yield in Brazil and United Kingdom was 0.85 (Costa et al., 2000), but decreased to 0.49 when considering two diverse countries, e.g., Kenya and United Kingdom (Ojango and Pollott, 2002). With focus on regions with similar management and climate characteristics, milk yield in North America was genetically closely correlated with milk yield in Western Europe (genetic correlations larger than 0.85) (Weigel et al., 2001). Additionally, different production systems, such as organic versus conventional (Nauta et al., 2006), small-scale versus large-scale (König et al., 2005), grazing versus confinement (Kearney et al., 2004), were considered as environmental descriptors in G×E studies. However, most of the genetic correlations between milk yields under varied production systems were close to 1, indicating only minor or absence of G×E. Further discrete environmental descriptors were milking system classifications (Mulder et al., 2004), herd classifications according to average production levels (Ruiz-Sánchez et al., 2007) and the herd fertility status (Craig et al., 2018).

### 1.6.2. Continuous environmental descriptors

Apart from multi-trait modeling to estimate genetic correlations between same traits from different discrete environments, enhanced RRM can be applied to estimate genetic (co)variance components for a longitudinal data structure along a continuous time and environmental scale. A prerequisite for RRM applications is a continuous covariate, reflecting changes in time or environment within the measuring period. Against this background, days in milk, as a classical covariate describing lactation stages, is included in RRM for the estimation of daily EBV for production traits (Jamrozik and Schaeffer, 1997). Furthermore, phenotypic herd descriptors, such as average herd protein yield and herd coefficients of variation for protein yield (Hayes et al., 2003) and bulk milk somatic cell count (Calus et al., 2006), were defined as continuous gradients in sire RRM. As an apparent indicator for climatic conditions, THI was considered in sire and animal RRM (Hayes et al., 2003; Brügemann et al., 2011), i.e., to study the effect of heat stress gradients on genetic variance components and to identify heat stress thresholds for production and functional traits. Additionally, a social-ecological descriptor, i.e., the survey stratification index (Hoffmann et al., 2017), reflecting the build-up density and the distance to

the city center, was a covariate in statistical models defined by Pinto et al. (2020). Pinto et al. (2020) aimed on stuying the influence of ecological gradients in combination with human-animal interactions on dairy cattle production and functional trait responses. In this thesis, genetic parameters, heritabilities and correlations for a broad trait pattern were estimated via sire RRM, considering phenotypic, genetic and genomic herd descriptors (as presented in the **original research paper 8**). Animal RRM were applied in a simulation study (**original research paper 1**), and in a study based on real test-day records (**original research paper 2**), aiming on the evaluation of predictive abilities of models with or without G×E.

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## 2. Publications

### 2.1. Original research paper 1

**Yin**, **T.**, E. C. G. Pimentel, U. König v. Borstel, and S. König:

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# Strategy for the simulation and analysis of longitudinal phenotypic and genomic data in the context of a temperature × humidity-dependent covariate

**T. Yin,* E. C. G. Pimentel,* U. König v. Borstel,† and S. König*[1]**
*Department of Animal Breeding, University of Kassel, 37213 Witzenhausen, Germany
†Department of Animal Breeding, Göttingen University, 37075 Göttingen, Germany

## ABSTRACT

A simulation study was conducted to evaluate the performance of genomic random regression models for the continuous environmental descriptor temperature-humidity index (THI). Statistically innovative aspects of the study included the combined simulation of both longitudinal phenotypic data representing the same trait in the course of THI and genomic data. The longitudinal trait was simulated (phenotypically expressed) at 5 different values of THI. For a moderate heritability trait, heritabilities were 0.30, 0.35, 0.40, 0.40, and 0.35 for THI of 15, 30, 45, 60 and 75, respectively. In a consecutive run, low heritabilities of 0.05, 0.1, 0.15, 0.15, and 0.10 were simulated, respectively. On the genomic level, simulation combined high and low linkage disequilibrium with 5,000-, 15,000-, and 50,000-SNP chip applications to simulate different scenarios of genomic architecture. With regard to data analyses, 2 strategies were applied to evaluate the accuracy of genomic predictions across THI, with special focus on the extreme ends of the environmental scale. In the first strategy, 100, 80, 50, or 20% of phenotypes at THI 75 were deleted randomly and the remaining data set was used to predict the breeding value at THI 75 for non-phenotyped, but genotyped cows. In the second strategy, 1,600 cows had complete information (genotypes and phenotypes) and 400 cows were genotyped, but with missing phenotypes for all THI. For the first strategy and without phenotypic observations at THI 75, accuracies of genomic predictions were lower than 0.34. When only 20% of cows had phenotypic records at THI 75, accuracies increased (∼0.60). Such a small proportion of phenotyped cows was sufficient to predict reliable genomic breeding values for cows without phenotypes for extreme THI. For the second strategy, also for low linkage disequilibrium combined with a low density 5,000-SNP chip, the average accuracy of genomic predictions was 0.52, which is substantially higher than accuracies based on pedigree relationships. From a practical perspective, genomic random regression models can be used to predict genomic breeding values for scarce phenotypes (e.g., novel traits) traits measured in extreme environments, or traits measured late in life, such as longevity.
**Key words:** genomic selection, genotype by environment interaction, random regression

## INTRODUCTION

Methods for dealing with longitudinal data in genetic evaluations have evolved from the use of repeatability models with permanent environmental effects or multiple-trait models with covariance matrices (Henderson, 1984) to random regression models (**RRM**; Schaeffer and Dekkers, 1994) with covariance functions (Kirkpatrick et al. 1990). The use of RRM for analyzing longitudinal production data are a standard in genetic evaluations for dairy cattle worldwide, because such models provide an overview of genetic parameters and breeding values across the whole lactation trajectory. Additionally, further applications of RRM to describe performances over a range of environments in reaction norm studies have been proposed. Such models are interesting with regard to genotype by environment interactions, where different environments can be defined on a continuous scale. Ravagnolo and Misztal (2000) estimated variance components for milk production traits at different levels of heat stress, defined by a temperature-humidity index (**THI**). In more recent studies, RRM were further elaborated by defining THI as an environmental covariate (Aguilar et al., 2009; Brügemann et al., 2011). The basic idea of RRM applications is to depict the physiological background or genetic mechanisms of traits in a quantitative genetic context, meaning that different genes are switched on or off with, for example, aging of the animal or with environmental changes. Substantial changes of both quantitative genetic parameters and gene expression profiles by inducing heat stress were shown in fertility

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[1]Corresponding author: sven.koenig@uni-kassel.de

traits of mice (Cammack et al., 2006, 2009). Genetic studies on heat stress in dairy cattle have an important practical background with regard to semen and livestock exports. For example, the German dairy cattle industry, and especially the dairy cattle breeding organization Masterrind GmbH (Verden, Germany), is strongly involved in exports of livestock and sire semen. Target countries include tropical countries located in Asia, Africa, and South America. In 2012, a total of 26,249 heifers were exported to these countries (DHV, 2013). The tropical and hot climates in the importing countries causes heat stress in the cows, especially when the THI rises above 72 (Bohmanova et al., 2005). In contrast, performance testing within Germany exhibits a shift from population-wide recording schemes toward so-called selected "contract herds" (Schierenbeck et al., 2011). Contract herds are characterized by superior feeding, management, and housing conditions, and by substantially lower THI levels realized by, for example, housing systems with integrated cooling techniques (Brügemann et al., 2012).

Classically, RRM applications (also when studying genotype by environment interactions) are based on longitudinal phenotypic records combined with genetic relationships from pedigree data. Nowadays, the availability of high-throughput genotyping technologies with decreasing costs encourages dairy cattle farmers worldwide to genotype an increasing percentage of cows, heifers, and female calves. Especially for novel traits, reliable, conventional EBV of bulls do not exist. Hence, basing genomic selection on calibration groups of cows might be a promising alternative (Buch et al., 2012). Examples include health traits (Pintus et al., 2013), and traits reflecting energy balance (Verbyla et al., 2010). Furthermore, Misztal et al. (2010) suggested the inclusion of genomic information to improve the accuracy of genetic evaluations of young animals for heat tolerance. Availability of cow genotypes combined with longitudinal phenotypic data enable the application of genomic RRM (**gRRM**) to estimate genomic breeding values (**GBV**) for scarcely recorded traits, or for environmental descriptors that are not or poorly represented in a data set. In this latter context, Suchocki and Szyda (2011) estimated SNP effects over time by applying a mixed model with orthogonal polynomials and genotyped animals for longitudinal growth data. An alternative might be the direct estimation of GBV using gRRM and BLUP. Simulations are a powerful tool to evaluate a broad variety of statistical procedures based on longitudinal phenotypic and genomic data and, in consequence, to study the effects of various scenarios on selection and mating schemes. Accuracies of genomic predictions strongly depend on technical parameters (e.g., size of calibration group and pattern of SNP chips), the quantity and quality of phenotypic data, quantitative genetic parameter estimates, and the genomic architecture of the trait. To our knowledge, no simulation package exists that simultaneously addresses those aspects for longitudinal data structures and directly provides true breeding values (**TBV**), GBV, and phenotypes in the course of a continuous environmental descriptor.

Consequently, the objectives of the present study were to (1) develop a framework for the simulation of longitudinal phenotypic data combined with high-throughput genotypes, (2) evaluate the performance of a gRRM in the context of reaction norms, and (3) investigate the accuracy of genomic predictions for cows that are poorly or not at all represented in the group of cows with records for environmental descriptors. For illustration and based on experiences from previous studies, the environmental descriptor THI was chosen, but applications to further problems will be discussed. The study was performed by varying the assumptions related to the genomic architecture of traits.

## MATERIALS AND METHODS

### *Simulation of Populations*

Populations were simulated using the software QMSim (Sargolzaei and Schenkel, 2009). With QMSim, the simulation process is divided in 2 stages. First, a historical population is simulated for several generations to generate a desired level of linkage disequilibrium (**LD**). In a second step, using animals from the last historical generation as founders, further recent populations are simulated for a desired number of generations. Within this second simulation step, population parameters can be varied to generate the appropriate population structure. In the present study, 2 different types of historical populations were simulated to create scenarios with either low or high LD. For both low-LD and high-LD scenarios, 10 recent generations were simulated based on the parameters as specified in Table 1.

Additionally, Table 1 summarizes the parameters of the simulated genome. The simulated genome consisted of 30 chromosomes of 100 cM each. The number of QTL per chromosome was set to 10 and QTL positions on the chromosome were randomly assigned. Effects of QTL alleles were drawn from a gamma distribution with a shape parameter 0.4. The number of QTL alleles at each locus was randomly assigned and was 2, 3, or 4. To achieve resemblance with different densities of SNP chips, 3 scenarios with respect to the number of markers on the genome were simulated. The simulation of 167, 500, and 1,667 biallelic markers per chromosome depicts applications with 5,000 (**5K**)-, 15,000 (**15K**)-,

**Table 1.** Parameters of the simulation process

| Item | Low LD[1] | High LD |
|---|---|---|
| Population structure | | |
| Historical population | | |
| Total generations (no.) | 1,640 | 2,620 |
| No. of animals in first generation | 4,040 | 2,000 |
| Bottleneck | No | Yes[2] |
| No. of animals in last generation | 4,040[2] | 4,040[3] |
| Current population | | |
| Generations | 10 | |
| No. of sires and dams | 40 and 4,000 | |
| No. of offspring per mate | 1 | |
| Probability for sex of the offspring | 0.5 | |
| Selection and mating designs | Random | |
| Replacement ratios for sires and dams (%) | 50 and 25 | |
| Culling criteria | Age | |
| Genomic parameters | | |
| No. of chromosomes | 30 | |
| Length of each chromosome (cM) | 100 | |
| No. of QTL per chromosome | 10 | |
| Effects of QTL alleles | Gamma (0.4) | |
| No. of QTL alleles | Random (2, 3, 4)[4] | |
| No. of biallelic markers per chromosome | 167; 500; 1,677 | |
| Maker and QTL mutation rate | $2.5 \times 10^{-5}$ (recurrent for markers) | |
| Crossover interference (cM) | 25 | |
| Position of markers and QTL | Random | |

[1]LD = linkage disequilibrium.

[2]Population size was 200 from generation 2,570 to 2,580.

[3]Forty males and 4,000 females.

[4]The number of QTL alleles at each locus was randomly assigned to be 2, 3, or 4.

and 50,000 (**50K**)-SNP chips, respectively. Both marker and QTL mutation rates were $2.5 \times 10^{-5}$, whereas recurrent mutation was only allowed for markers. The crossing-over interference was defined as 25 cM (i.e., a random placement of crossing over along chromosomes can be interrupted if the distance between a pair of a crossing over is smaller than 25 cM).

### *Simulation of Longitudinal Data*

The QMSim output, which included QTL genotypes, QTL effects, and SNP markers, was modified by our own programming to produce longitudinal data records for 2,000 cows in the last generation. In total, 300 QTL controlling the trait of interest (i.e., heat tolerance per se or performances at different heat stress levels) were randomly divided into 10 groups, with an even group size of 30 QTL. True breeding values of cows in 10 different groups were based on the 30 different QTL in each group and were calculated using the corresponding QTL effects as produced from the QMSim program. Following the protocol of Brügemann et al. (2011), the environmental continuous descriptor THI varied from 15 to 75 in increments of 15. To model changes in gene expression in the course of THI, only QTL in groups 1 to 6 were assigned to express at THI 15, indicating that TBV of 2,000 cows at THI 15 (TBV_THI15) were the sum of effects for each QTL in groups 1 to 6. At THI 30, only QTL in groups 2 to 7 contributed to TBV_THI30, and so on (Table 2). This simulation strategy resulted in 5 groups of overlapping QTL (i.e., 150 QTL) between adjacent THI levels. For example, QTL in groups 2 to 6 expressed simultaneously at THI 15 and THI 30. Even the 2 most extreme THI levels (THI 15 and THI 75) had 2 groups of QTL in common (groups 5 and 6). Simulation was done in this way to smooth the correlations across the THI levels. Furthermore, this simulation strategy reflects the fact that the trait has some basic genetic background effecting its phenotypic expression also with environmental changes. Consequently, the true genetic correlations between THI levels were determined by the number of overlapping QTL groups, which depicts the basic idea and flexibility of our simulation strategy.

Phenotypes were created by adding a residual to TBV at THI 15 to 75 according to a set of defined heritabilities. Heritabilities for the 5 chosen THI (15, 30, 45, 60, and 75) were 0.30, 0.35, 0.40, 0.40, and 0.35, respectively, for a moderate heritability trait, and 0.05, 0.10, 0.15, 0.15, and 0.10, respectively, in a consecutive run for a low heritability trait. In total, the number of phenotypic records for the 2,000 cows was 10,000, indicating that each cow had 5 repeated measurements (i.e., the same trait expressed at 5 different THI). Lon-

**Table 2.** Expression of groups of QTL at different temperature-humidity index (THI) levels for mimicking the physiological background of gene expression

| | QTL group | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| THI | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 15 | X[1] | X | X | X | X | X | | | | |
| 30 | | X | X | X | X | X | X | | | |
| 45 | | | X | X | X | X | X | X | | |
| 60 | | | | X | X | X | X | X | X | |
| 75 | | | | | X | X | X | X | X | X |

[1]X = QTL expressed in the group.

gitudinal data records are characterized by phenotypic and genetic relationships along the trajectory of the continuous environmental descriptor THI. Genetic and phenotypic correlations between 5 different THI averaged across 10 replicates are listed in Table 3 for the moderate-heritability scenario and in Table 4 for the low-heritability scenario. Higher genetic correlations for protein yield between adjacent THI than for very different THI were also found by Brügemann et al. (2011) and reflect the physiological and practical background of our simulation.

### Statistical Model

A gRRM was applied to analyze the simulated longitudinal data using the AI-REML algorithm and applying the DMU package (Madsen and Jensen, 2010). In matrix notation, the statistical model was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{p} + \mathbf{e},$$

where $\mathbf{y}$ = vector of phenotypic observations for cows, $\mathbf{b}$ = vector of fixed regressions on THI using third-order Legendre polynomials, $\mathbf{a}$ = vector of additive genetic effects for random regression coefficients on THI using third-order Legendre polynomials, $\mathbf{p}$ = vector of permanent environmental effects, $\mathbf{e}$ = vector of random residual effects, and $\mathbf{X}$, $\mathbf{Z}_1$, and $\mathbf{Z}_2$ = incidence matrices for $\mathbf{b}$, $\mathbf{a}$, and $\mathbf{p}$, respectively. The genetic relationship matrix between individuals was either built via pedigrees including 10 generations ($\mathbf{A}$) or via SNP data of cows ($\mathbf{G}$). In the following, scenarios based on genomic data are labeled G_*, where the asterisk (*) specifies the level of LD and the size of the SNP chip. The pedigree-based scenario is labeled **A**. The **G** matrix was computed from the Gmatrix program (Su and Madsen, 2011) based on the method proposed by VanRaden (2008). Markers with minor allele frequency lower than 0.05 were deleted. A small value (0.01) was added to the diagonal of the **G** matrix to ensure that the matrix was positive definite.

For all scenarios, results from the gRRM were compared by applying a relatively simple genomic repeatability model (**gRM**). In matrix notation, the gRM was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{p} + \mathbf{e},$$

where $\mathbf{b}$ = vector of fixed effects of THI levels and $\mathbf{a}$ = vector of additive genetic effects. Other effects were identical to those of the gRRM.

### Accuracy of Genomic Predictions

Conventional EBV and GBV in the course of the environmental descriptor were calculated by multiplying random regression coefficients for additive genetic effects for each cow with corresponding Legendre polynomials for THI 15, 30, 45, 60, and 75. Accuracies of estimates were correlations between TBV and GBV (or EBV). For calculating the accuracy of genomic predic-

**Table 3.** Heritabilities (diagonal), genetic (above diagonal), and phenotypic (below diagonal) correlations of the simulated longitudinal data between different temperature-humidity indices (THI)[1]

| | THI | | | | |
|---|---|---|---|---|---|
| THI | 15 | 30 | 45 | 60 | 75 |
| 15 | 0.30 | 0.86 | 0.69 | 0.54 | 0.38 |
| 30 | 0.28 | 0.35 | 0.83 | 0.68 | 0.53 |
| 45 | 0.24 | 0.32 | 0.40 | 0.84 | 0.69 |
| 60 | 0.19 | 0.25 | 0.35 | 0.40 | 0.85 |
| 75 | 0.13 | 0.18 | 0.27 | 0.32 | 0.35 |

[1]Simulation of a moderate-heritability trait.

**Table 4.** Heritabilities (diagonal), genetic (above diagonal), and phenotypic (below diagonal) correlations of the simulated longitudinal data between different temperature-humidity indices (THI)[1]

| | THI | | | | |
|---|---|---|---|---|---|
| THI | 15 | 30 | 45 | 60 | 75 |
| 15 | 0.05 | 0.86 | 0.69 | 0.54 | 0.38 |
| 30 | 0.05 | 0.10 | 0.83 | 0.68 | 0.53 |
| 45 | 0.06 | 0.10 | 0.15 | 0.84 | 0.69 |
| 60 | 0.05 | 0.08 | 0.13 | 0.15 | 0.85 |
| 75 | 0.01 | 0.05 | 0.08 | 0.10 | 0.10 |

[1]Simulation of a low-heritability trait.

tions for cows without phenotypes, 2 strategies of data analysis were applied. In the first strategy, 100, 80, 50, or 20% of records only at THI 75 were deleted randomly and the remaining data set was used to predict GBV (or EBV) for non-phenotyped cows at THI 75. In a second strategy, complete information (genotypes and phenotypes) was available from 1,600 cows (training set), and phenotypes were missing from 400 cows (validation set) for all THI. Within this second strategy, 2 approaches were simulated to allocate cows to the 2 sets. In the first approach, 1,600 cows were randomly selected as reference animals and used to predict GBV (or EBV) for the 400 cows without phenotypes. An alternative approach to allocate cows in training and validation sets was accomplished by mimicking low relationships between candidates in both groups. The alternative approach implied that the training set only included daughters of 8 sires (400 cows), and daughters from the other 32 sires (1,600 cows) were represented in the validation set. The 2 different strategies and the 2 approaches within the second strategy for allocating cows in training and validation sets are illustrated in Figure 1.

## RESULTS AND DISCUSSION

### Genomic Architecture

Degree of LD between all possible SNP pairs was measured by using the squared correlation coefficient ($\mathbf{r^2}$; Hill and Robertson, 1968). In total, 2,000 cows with genomic information were used to compute $r^2$. The bottleneck in the historical population was a crucial factor influencing LD between markers. Linkage disequilibrium strongly determines the genomic architecture of traits, which has a substantial effect on (1) the choice of the method for genomic evaluations and (2) the reliability of its outcome (e.g., Daetwyler et al., 2010). The average $r^2$ from 1 of the 30 chromosomes across 10 replicates between SNP pairs for both the low LD and high LD scenarios combined with 5K-, 15K-, and 50K-SNP chip applications are plotted against a map distance of up to 2 Mb (Figure 2). Generally, average $r^2$ for both high- and low-LD scenarios decreased with an increase in map distances between SNP. We observed substantial declines with regard to average $r^2$ for distances in the range from 0 to 0.8 Mb, but the declines were low to moderate for distances ranging between 0.8 and 2.0 Mb. Such an exponential decrease for $r^2$ was shown in several studies (e.g., Jiménez-Montero et al., 2013). The average $r^2$ for high-LD scenarios were larger than for low-LD scenarios, especially for a small distance between 2 SNP. However, if the distance between markers was larger than 5.0 Mb, no difference in $r^2$ was found between high-LD and low-LD scenarios. The average $r^2$ was very close to zero (0.005) when distances were 100 Mb.

### Accuracies of Breeding Values for Cows With Phenotypes

Accuracies of GBV of cows with phenotypes at all THI levels were calculated for both scenarios (high and

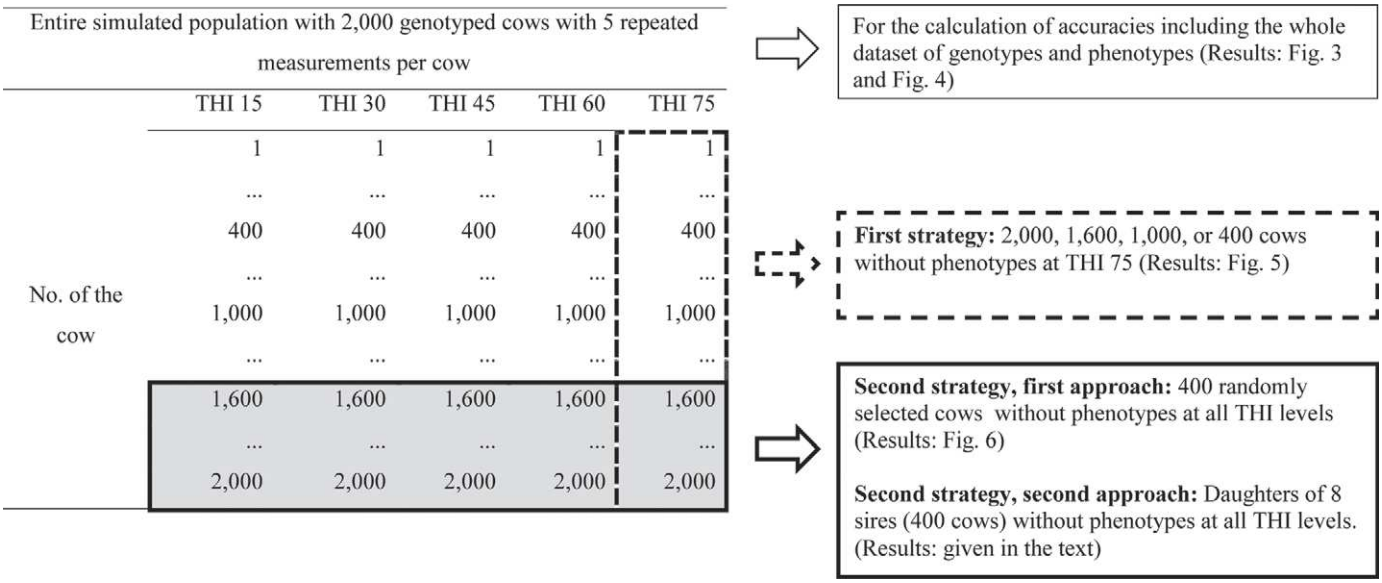


**Figure 1.** Strategies and approaches within strategies for defining subsets for genomic prediction with genomic random regression models (cows given in the dashed-line box and in the gray box indicate the animals without phenotypes for the 2 different strategies). THI = temperature-humidity index.

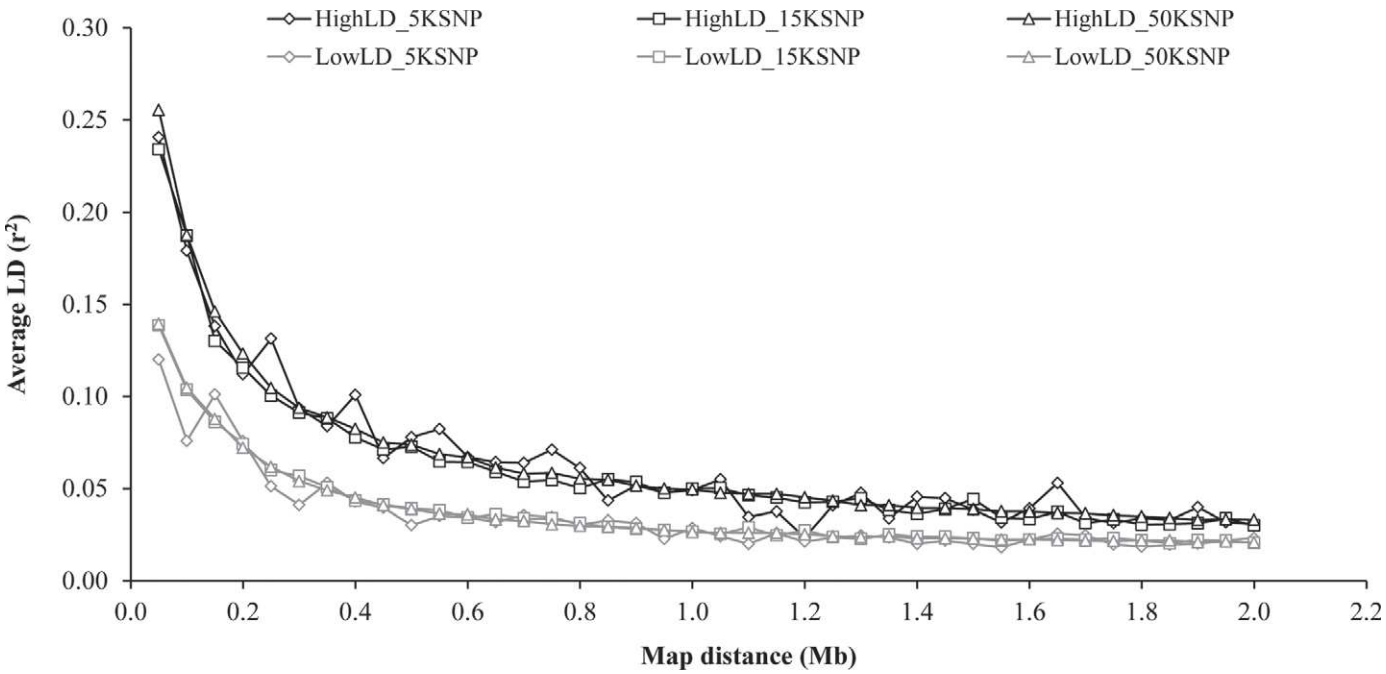


**Figure 2.** Average linkage disequilibrium [LD; squared correlation coefficient ($r^2$)] between SNP markers dependent on their map distance for the different sets of simulated marker data. 5K = 5,000; 15K = 15,000; 50K = 50,000.

low LD) combined with different sizes of SNP chips (5K, 15K, and 50K). Average accuracies from 10 replicates for the longitudinal trait with moderate heritability using the complete data set of 2,000 cows with phenotypes and genotypes are shown in Figure 3. Generally, accuracies of GBV and EBV increased with increasing heritabilities and were highest for a heritability of 0.40 at THI 45. Interestingly, accuracies at THI 30 are marginally higher than at THI 75 even though the same heritability ($h^2 = 0.35$) was assumed. To explain this phenomenon, a trait with a constant heritability ($h^2 = 0.35$) in the course of THI was simulated and accuracies for the 2 scenarios G_HighLD_15KSNP and A (where G = scenario based on the genetic relationship matrix using the SNP data of cows and A = scenario based on the pedigree relationship matrix) were calculated. For both scenarios, and assuming identical heritabilities along the trajectory, accuracies of predictions were highest at THI 45. An explanation might be that phenotypic records at THI 45 were genetically highly correlated with records measured at remaining THI (the lowest genetic correlation was 0.68). In contrast, for GBV (or EBV) estimated at THI 15, only data from THI in close proximity to THI 15 (THI 30 and 45) were highly correlated with data from THI 15 (genetic correlation >0.68). Records at the upper end of the environmental scale (THI 60 and 75) only showed moderate genetic correlations of 0.54 and 0.38, respectively (Table 3).

Accuracies of GBV for scenarios based on genomic relationship matrices were always higher compared with EBV from the pedigree-based scenario A, except for low LD combined with a low-density SNP chip (G_LowLD_5KSNP; Figure 3). The highest accuracy was realized in scenario G_HighLD_50KSNP with a value of 0.82 at THI 45 and 60. For genomic scenarios, accuracies of GBV increased with increasing LD and increasing size of the SNP chip. A strong and positive relationship between LD and accuracy of GBV was observed in previous studies (e.g., by Zhong et al., 2009). Due to the fact that more markers and QTL are in LD, more markers capture a higher proportion of genetic variance of the trait (Goddard, 2009). Additionally, higher marker density increased the $r^2$ between adjacent markers and the accuracy of genomic prediction (Meuwissen, 2009). As one example in our study, the average $r^2$ for the scenario G_HighLD_50KSNP was 0.26, but $r^2$ was only 0.11 for the scenario G_HighLD_5KSNP. The corresponding averaged accuracies of genomic predictions for the 2 scenarios were 0.79 and 0.74, respectively.

Figure 4 depicts accuracies of predictions for different levels of LD and marker density at 5 THI for the low-heritability trait. As expected, accuracies were lower compared with results for the moderate-heritability trait (Figure 3), but revealing identical tendencies with regard to LD and the size of SNP chips. Especially for the low-heritability trait in the range from THI 15 to 45 (Figure 4), genomic scenarios with high LD achieved

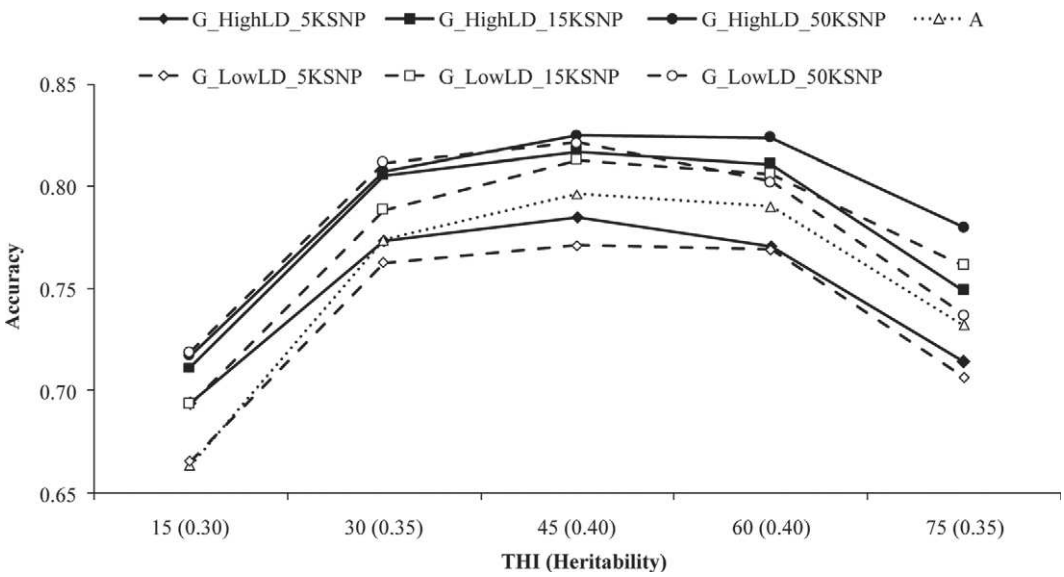


**Figure 3.** Average accuracies of genomic predictions for a longitudinal trait with heritabilities of 0.30, 0.35, 0.40, 0.40, and 0.35 for temperature-humidity indices (THI) of 15, 30, 45, 60, and 75, respectively. Different scenarios include variation in SNP density [5,000 (5K), 15,000 (15K), and 50,000 (50K)] and linkage disequilibrium (LD). G = scenario based on the genetic relationship matrix using the SNP data of cows; A = scenario based on the pedigree relationship matrix.

substantially higher accuracies of prediction compared with the pedigree-based scenario. Buch et al. (2012) also found that genotyping of cows simultaneously increased accuracies of genomic prediction for traits with low heritability. When basing within-herd replacement decisions of cows on GBV instead on pedigree indices, accuracy of selection and genetic merit of females will be improved with an associated positive effect on farm economy (Weigel et al., 2012).

For the low-heritability trait, the pedigree-based RRM, but especially the more complex gRRM, revealed some convergence problems (i.e., longer computing time was required to meet convergence criteria). Hence, we conclude that more observations are required when applying gRRM to low-heritability traits. Yin et al. (2012) applied RRM to estimate genetic parameters by DIM and by parity for low-heritability functional traits comprising a comparatively small data set of 1,283 cows from low-input systems in Switzerland. In some cases, statistical models not only failed to converge, but also estimates of heritabilities or genetic correlations were extraordinarily high at the extreme ends of the continuous time scale.

### *Accuracies of Breeding Values for Cows Without Phenotypes*

Figure 5 shows accuracies of breeding values for non-phenotyped cows by altering percentages of cows with phenotypes at an extreme end of the continuous environmental scale (THI 75). For the moderate-heritability scenario, and without phenotypic records at THI 75, accuracies of predictions at THI 75 were generally low and, on average, at a value of 0.22 (average of all scenarios) with corresponding high standard deviations in the range from 0.19 to 0.33 within scenarios. However, for 20% of phenotyped cows at THI 75, accuracies of predictions substantially increased to a moderate value of 0.60, by decreasing standard deviations (within scenarios, the standard deviation decreased by factor 5). An average accuracy of prediction of 0.69 was realized for 80% of the 2,000 cows with phenotypic records at THI 75 and with the 15K SNP chip used for genotyping. Interestingly, for the scenario without phenotypes at THI 75, accuracies of predictions from the gRM were higher than from the gRRM. For example, for the scenario G_HighLD_15KSNP, accuracies were 0.56 and 0.33, respectively. In contrast with 20% or more of phenotyped cows at THI 75, gRRM were superior to gRM. Again, for the scenario G_HighLD_15KSNP, accuracy from the gRRM was 0.63, and 0.57 from the gRM. Generally, standard deviations of accuracies from the gRM were larger than standard deviations from gRRM applications.

According to the heat stress studies conducted by Brügemann et al. (2012) in Germany, only a limited number of genotyped heifers or cows had phenotypic performances in environments with extremely high THI. Based on the results from our present study, only a small proportion of phenotyped cows (i.e., 20%) in

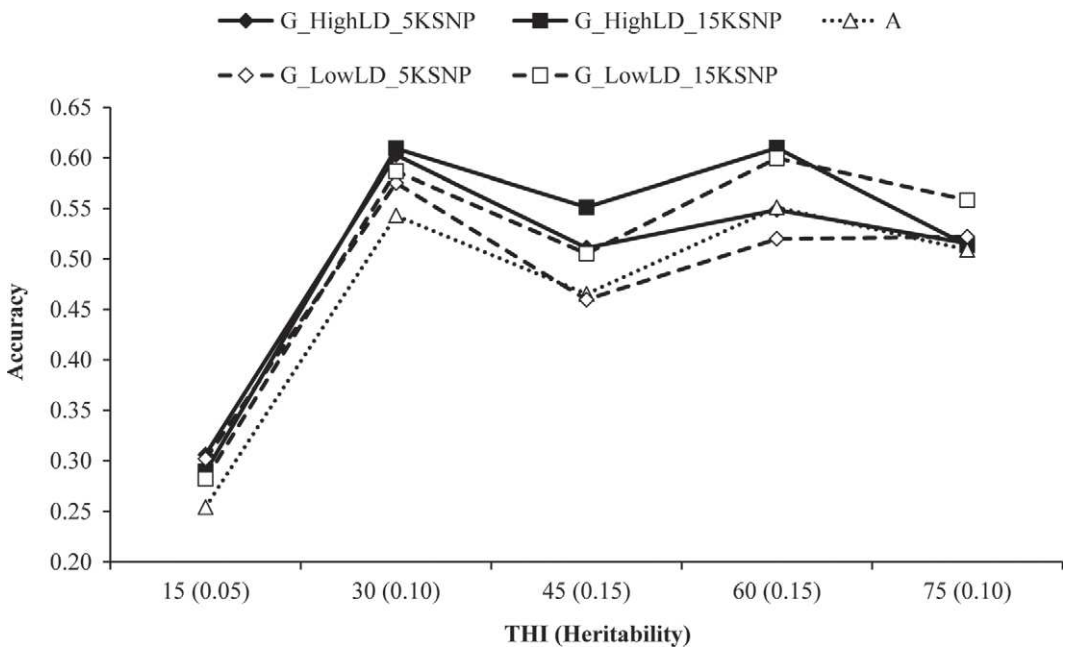


**Figure 4.** Average accuracies of genomic predictions for a longitudinal trait with heritabilities of 0.05, 0.10, 0.15, 0.15, and 0.10 for temperature-humidity indices (THI) of 15, 30, 45, 60, and 75, respectively. Different scenarios include variation in SNP density [5,000 (5K) and 15,000 (15K)] and linkage disequilibrium (LD). G = scenario based on the genetic relationship matrix using the SNP data of cows; A = scenario based on the pedigree relationship matrix.

environments representing heat stress (i.e., THI 75) is required to predict reliable GBV of cows without phenotypes. Without phenotypic records at THI 75, the maximal accuracy amounts to 0.33, indicating that RRM or gRRM cannot predict the genetic variance of the trait accurately when there is a complete lack of phenotypes at the extreme ends of a continuous environmental scale. A further practical application of gRRM might be the prediction of GBV for longevity of genotyped heifers or other traits measured late in a dairy cow's life. Such a general application of RRM for predicting longevity was suggested by Schaeffer (2004).

Figure 6 depicts accuracies of predictions for the pedigree-based and for the genomic scenarios with 1,600 cows in a calibration set (i.e., genotypes and phenotypes were available for all THI), and 400 cows in the prediction set (i.e., cows with genotypes but without phenotypes). The scenario high LD using the 15K SNP chip (G_HighLD_15KSNP) provided the highest accuracy of genomic predictions across THI. Average accuracies across replicates and THI were 0.54 for the scenario high LD with the 5K SNP chip, 0.58 for the scenario high LD with the 15K SNP chip, 0.49 for scenario A, 0.52 for the scenario low LD with the 5K SNP chip, and 0.56 for the scenario low LD with the 15K SNP chip. Among the 5 scenarios, again the pedigree-based predictions were less accurate compared with genomic predictions, even compared with low LD in combination with the low-density 5K SNP chip (accuracy of genomic prediction ranged between 0.49 and 0.55). Not only gRRM, but also genomic multiple-trait models increase the accuracy of genomic predictions compared with genomic univariate models (Tsuruta et al., 2011), especially when multiple traits are strongly correlated. For an environmental descriptor with only a few environmental levels, also a genomic multiple-trait model can be applied, but not for a continuous environmental descriptor with more levels at closer intervals (as also valid for THI, in practice).

In addition to genetic architecture and marker density, the level of genetic relationships between cows in the defined calibration set and cows in the prediction set is a crucial factor influencing the accuracy of genomic predictions in gRRM. The genotyped 2,000 cows in this study were progeny from 40 sires, with an average of 50 daughters per sire. For the first approach of the second strategy (Figure 1), each sire had, on average, 10 daughters in the calibration set and 40 daughters in the training set. Therefore, cows in the prediction set were genetically related to their half-sibs in the prediction set and these close relationships might have increased the accuracy of genomic prediction. To verify accuracies caused by LD and marker density only, a large genetic distance between both groups should be achieved (Bolormaa et al., 2010). This distance was maximized by deleting phenotypes of daughters from 8

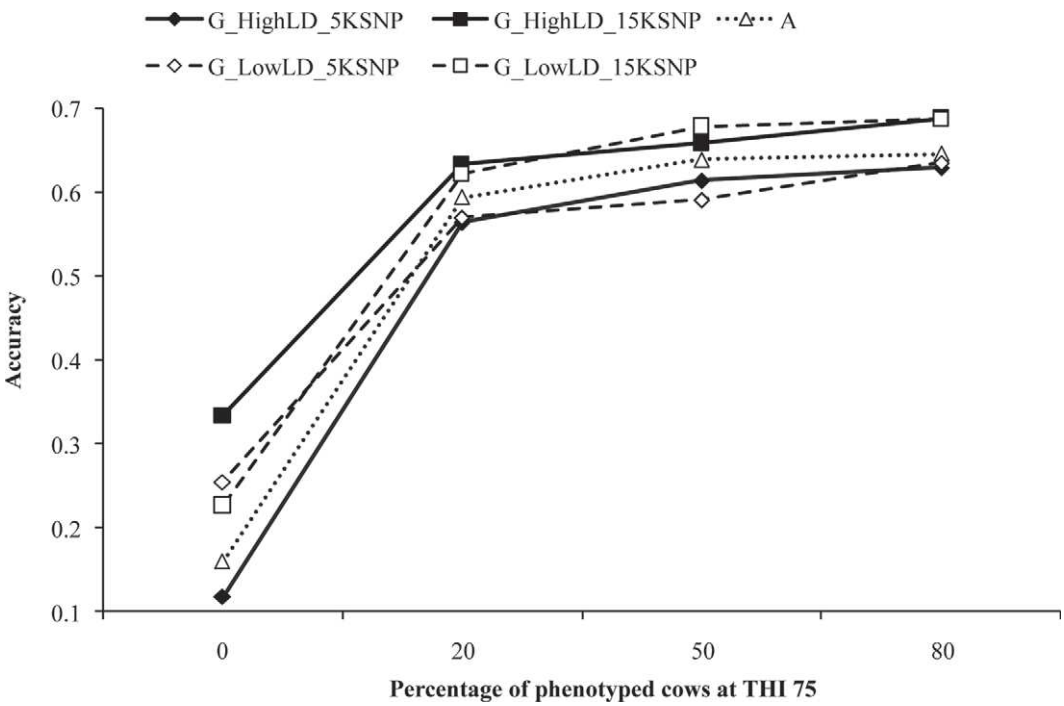


**Figure 5.** Average accuracies of genomic predictions for non-phenotyped cows at a temperature-humidity index (THI) of 75 for 0, 20, 50, and 80% of cows with phenotypic records at THI 75 with heritabilities of 0.30, 0.35, 0.40, 0.40, and 0.35 for THI 15, 30, 45, 60, and 75, respectively. Different scenarios include variation in SNP density [5,000 (5K) and 15,000 (15K)] and linkage disequilibrium (LD). G = scenario based on the genetic relationship matrix using the SNP data of cows; A = scenario based on the pedigree relationship matrix.

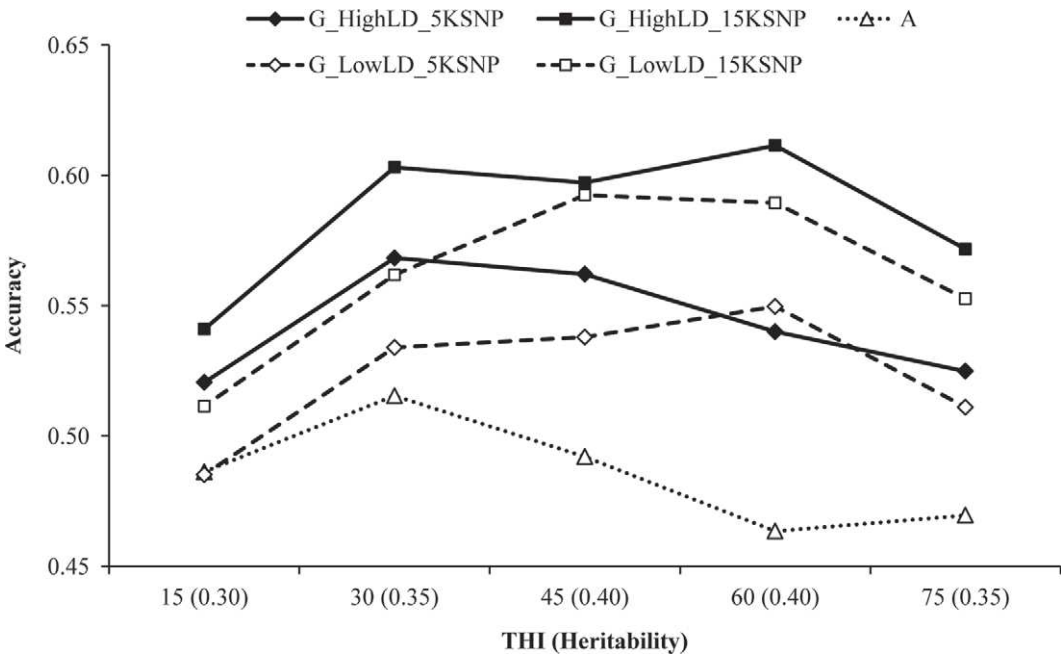


**Figure 6.** Average accuracies of genomic predictions for the scenarios with 400 cows in the prediction set and 1,600 cows in the calibration set with heritabilities of 0.30, 0.35, 0.40, 0.40, and 0.35 for temperature-humidity indices (THI) of 15, 30, 45, 60, and 75, respectively. Different scenarios include variation in SNP density [5,000 (5K) and 15,000 (15K)] and linkage disequilibrium (LD). G = scenario based on the genetic relationship matrix using the SNP data of cows; A = scenario based on the pedigree relationship matrix.

**Table 5.** Average pedigree- and genomic-based relationships for cows within the calibration and the prediction population, and between the 2 populations[1]

| | Relationship | | | | | |
|---|---|---|---|---|---|---|
| Scenario[2] | Calibration[3] | Calibration[4] | Prediction[3] | Prediction[4] | Between[3] | Between[4] |
| G_LowLD_5KSNP | 0.0008 | 0.0022 | 0.0045 | 0.0285 | −0.0005 | −0.0064 |
| G_LowLD_15KSNP | 0.0008 | 0.0023 | 0.0045 | 0.0291 | −0.0005 | −0.0067 |
| A | 0.0348 | 0.0362 | 0.0382 | 0.0616 | 0.0335 | 0.0277 |
| G_HighLD_5KSNP | 0.0008 | 0.0022 | 0.0044 | 0.0283 | −0.0005 | −0.0064 |
| G_HighLD_15KSNP | 0.0008 | 0.0022 | 0.0044 | 0.0287 | −0.0005 | −0.0068 |

[1]Averages are from 10 replicates.

[2]G = scenario based on the genetic relationship matrix using the SNP data of cows; A = scenario based on the pedigree relationship matrix; LD = linkage disequilibrium; 5K = 5,000; 15K = 15,000.

[3]Four hundred cows were randomly allocated to the prediction population; the remaining cows (1,600) were assigned to the calibration population.

[4]Daughters of 8 sires were allocated to the prediction population; the remaining cows were assigned to the calibration population.

sires. The cows from the remaining 32 sires comprised the training set. Hence, the average pedigree-based genetic relationships between cows in the calibration sets and cows in the prediction sets were lower, and ranged from 3.35 to 2.77% (Table 5). The accuracy of predictions for the 5 scenarios decreased, on average, by 25%. In detail, the accuracy was 0.34 for scenarios with pedigree information, 0.44 for G_HighLD_15KSNP, and 0.44 for G_LowLD_15KSNP. Corresponding accuracies were 0.49, 0.59, and 0.56 when 400 cows were randomly allocated to the prediction set. Also for gRRM, the results indicate that genetic relationships between cows in the calibration set and cows in the prediction set played an important role with regard to realized accuracies of predictions. However, accuracies that result from genetic relationships will erode in the long term, and only the effects of SNP in LD with QTL are persistent across populations and generations (Hayes et al., 2009).

Moderately accurate genomic predictions with gRRM also for cows that are completely non-phenotyped (i.e., without phenotypes across THI) allow further improvements with regard to on-farm selection strategies. The availability of a cheap low-density SNP chip implies an extension of genotyping from preselected bull dams toward cohorts of heifers or young cows in commercial dairy cattle herds (e.g., Achler, 2013). Additionally, efficient imputing strategies for totally ungenotyped animals that use information from genotyped relatives are developed for practical application (Pimentel et al., 2013). Application of those imputing techniques will enlarge the pool of genotyped animals while keeping costs constant. Hence, the lack of phenotypes is the most limiting factor for novel traits that are difficult or expensive to measure (e.g., traits related to health, product quality, or animal welfare). Hence, reliable GBV can be predicted when genotyping cows on a large scale, but only phenotyping a subset of the genotyped cows for novel traits. Such a scenario might be relevant, for example, for longitudinal health traits as recorded in contract herds (Gernand et al., 2012).

## CONCLUSIONS

In the present study, first we developed a strategy for simulating longitudinal phenotypic records along with marker information. This strategy is based on differentiated gene or QTL expression on a continuous environmental scale and might be helpful for future evaluations of breeding strategies based on longitudinal data sets. From a methodological point of view, results from this study revealed higher accuracies of predictions when replacing the traditional pedigree-based genetic relationship matrix with the realized genomic relationship matrix in RRM applications. The simultaneous use of genetically correlated longitudinal data in gRRM can predict genetic values of animals without phenotypes. Only a small proportion of cows (i.e., 20%) with phenotypes at the extreme ends of an environmental scale (here, THI 75) is required to predict GBV of non-phenotyped cows at THI 75. For this scenario, accuracies of predictions of GBV from gRRM for completely non-phenotyped cows were of moderate size and ranged between 0.50 and 0.60. For a minimized genetic relationship between the calibration and the prediction population, accuracies of genomic predictions decreased for various scenarios (scenarios included variations in LD and of SNP density) by 20 to 30%.

## ACKNOWLEDGMENTS

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## 2.2. Original research paper 2

Bohlouli, M., S. Alijani, S. Naderi, **T. Yin**, and S. König:

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# Prediction accuracies and genetic parameters for test-day traits from genomic and pedigree-based random regression models with or without heat stress interactions

**M. Bohlouli,[1] S. Alijani,[2] S. Naderi,[1] T. Yin,[1] and S. König[1]***
[1]Institute of Animal Breeding and Genetics, Justus-Liebig-University Gießen, 35390 Gießen, Germany
[2]Department of Animal Science, University of Tabriz, 51666 Tabriz, East Azarbaijan, Iran

## ABSTRACT

The aim of this study was to compare genetic (co)variance components and prediction accuracies of breeding values from genomic random regression models (gRRM) and pedigree-based random regression models (pRRM), both defined with or without an additional environmental gradient. The used gradient was a temperature-humidity index (THI), considered in statistical models to investigate possible genotype by environment (G×E) interactions. Data included 106,505 test-day records for milk yield (MY) and 106,274 test-day records for somatic cell score (SCS) from 12,331 genotyped Holstein Friesian daughters of 522 genotyped sires. After single nucleotide polymorphism quality control, all genotyped animals had 40,468 single nucleotide polymorphism markers. Test-day traits from recording years 2010 to 2015 were merged with temperature and humidity data from the nearest weather station. In this regard, 58 large-scale farms from the German federal states of Berlin-Brandenburg and Mecklenburg-West Pomerania were allocated to 31 weather stations. For models with a THI gradient, additive genetic variances and heritabilities for MY showed larger fluctuations in dependency of DIM and THI than for SCS. For both traits, heritabilities were smaller from the gRRM compared with estimates from the pRRM. Milk yield showed considerably larger G×E interactions than SCS. In genomic models including a THI gradient, genetic correlations between different DIM × THI combinations ranged from 0.26 to 0.94 for MY. For SCS, the lowest genetic correlation was 0.78, estimated between SCS from the last DIM class and the highest THI class. In addition, for THI × THI combinations, genetic correlations were smaller for MY compared with SCS. A 5-fold cross-validation was used to assess prediction accuracies from 4 different models. The 4 different models were gRRM and pRRM, both modeled with or without G×E interactions. Prediction accuracy was the correlation between breeding values for the prediction data set (i.e., excluding the phenotypic records from this data set) with respective breeding values considering all phenotypic information. Prediction accuracies for sires and for their daughters were largest for the gRRM considering G×E interactions. Such modeling with 2 covariates, DIM and THI, also allowed accurate predictions of genetic values at specific DIM. In comparison with a pRRM, the effect of a gRRM with G×E interactions on gain in prediction accuracies was stronger for daughters than for sires. In conclusion, we found stronger effect of THI alterations on genetic parameter estimates for MY than for SCS. Hence, gRRM considering THI especially contributed to gain in prediction accuracies for MY.

**Key words:** genotype by environment interaction, temperature-humidity index, random regression model, genomic prediction

## INTRODUCTION

In the global dairy cattle industry, daughter records of bulls are available in various environments and countries because of the widespread use of AI. Environmental differences might contribute to daughter record variations, consequently resulting in re-rankings of bulls across environments (Hammami et al., 2009; Hayes et al., 2016). These are the principles of a genotype by environment (**G×E**) interaction, meaning that different genotypes react differently across environments (Falconer and Mackay, 1996). Generally, in quantitative genetic studies, a genetic correlation lower than 0.80 between the same trait measured in 2 discrete environments indicates G×E interaction (Robertson, 1959). Challenging environmental impact, especially climate change and associated heat stress response, might cause re-rankings of sires under different climatic conditions.

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*Corresponding author: sven.koenig@agrar.uni-giessen.de

As a consequence, to be competitive in a broad range of climates worldwide, international dairy cattle breeding programs should consider G×E interaction effects in genetic evaluations (Ravagnolo and Misztal, 2000). Some countries (e.g., Australia) routinely implement genomic and genetic evaluations for heat tolerance (Nguyen et al., 2018).

In the past decade, several studies analyzed G×E interactions for milk production traits using either pedigree-based relationship matrices (**pRRM**; Calus et al., 2006; Hammami et al., 2013; Carabaño et al., 2016) or genomic relationship matrices (**gRRM**; Haile-Mariam et al., 2015; Tiezzi et al., 2017). To quantify differences in gene expressions in various environments, genomic multiple-trait models (Haile-Mariam et al., 2015; Yao et al., 2017) and reaction norm or random regression models (**RRM**; Macciotta et al., 2017; Tiezzi et al., 2017) were applied. Yao et al. (2017) and Tiezzi et al. (2017) demonstrated the advantages of statistical models with interaction effects for the estimation of environment-specific genomic parameters. Accordingly, also in simulations, genomic predictions had improved accuracies when considering specific QTL in different environments (Bohlouli et al., 2017).

Random regression models allow the estimation of genetic (co)variance components and breeding values over the whole trajectory of a time-dependent (e.g., DIM) or environment-dependent (e.g., temperature-humidity indices; **THI**) covariate. Modeling the effect of a genotype as a function of time and environment (Bohmanova et al., 2007; Brügemann et al., 2011) implies the detection of G×E interactions via differences in genetic (co)variance components for different combinations of DIM with THI. In this regard, Ravagnolo and Misztal (2000) found considerable genetic variances for heat tolerance when applying an RRM to production traits and including a function for THI. Furthermore, specific genetic parameters or breeding values for distinct heat stress levels (Brügemann et al., 2011; Nguyen et al., 2016) are suitable indicators for the selection of optimal progeny testing environments (Schierenbeck et al., 2011) or for the selection of herds to be included in cow training sets for genomic selection (Naderi et al., 2018). However, the effect of THI combined with a large data set of genotyped cows from commercial large-scale production herds on possible G×E interactions, and on accuracies of genomic predictions, needs deeper analyses. Therefore, the objective of the present study was to apply RRM with gRRM and pRRM and to study the effect of models with THI gradients on (1) estimates for genetic (co)variance components, (2) possible G×E interactions, and (3) accuracies of genomic predictions.

## MATERIALS AND METHODS

### Phenotypes

Data were first-lactation test-day records for milk yield (**MY**) and SCC (recording years 2010–2015) from 12,331 genotyped Holstein dairy cows kept in 58 large-scale test herds. Herds were located in the region of former East Germany (i.e., in the federal states of Berlin-Brandenburg and Mecklenburg-Western Pomerania). Age at first calving ranged from 20 to 39 mo. Days in milk were restricted between 5 and 305 d. Each cow had at least 5 test-day records, and a minimum of 5 records were defined for each herd test-date. Test-day SCC was transformed to SCS by $\text{SCS} = \log_2\left(\frac{\text{SCC}}{100,000}\right) + 4$. A constant 4 was added to avoid the problem of negative values in the data (Martins et al., 2011). After data editing, 106,274 test-day records were available for SCS and 106,505 test-day records were available for MY. The SCS ranged from 0.16 to 10.47, and MY ranged from 2.0 to 61.5 kg.

### Genotypes and Pedigrees

A total of 5,104 animals, including 4,973 cows and 131 sires, were genotyped with the Illumina BovineSNP50 v2 BeadChip (Illumina, San Diego, CA). A further set of 7,749 animals, including 7,358 cows and 391 sires, were genotyped with the Illumina Bovine Eurogenomics 10K low-density chip (the so-called Euro10K LD chip; see Reents, 2014). The animals genotyped with 10K were further imputed to 50K using the procedure for official national genetic evaluations as implemented by project partner VIT Verden (Segelke et al., 2012). After imputation, 45,613 SNP were available from 522 genotyped sires and 12,331 genotyped cows. The SNP quality controls were performed using the preGSf90 program (Aguilar et al., 2010). First, we discarded 3,581 SNP with minor allele frequency lower than 0.05. According to Wiggans et al. (2009), a difference between observed and expected heterozygous frequencies larger than 0.15 indicates a departure from Hardy–Weinberg equilibrium. In this regard, we excluded 2 SNP from the ongoing analyses. Furthermore, according to Wiggans et al. (2009), we discarded 696 highly correlated SNP and 866 SNP located on the X and Y chromosomes. Finally, 40,468 SNP were considered for all genomic analyses.

Pedigree was traced back as far as possible (back to founders born in 1965) to increase across-herds genetic connectedness. The pedigree file included 48,977 animals (i.e., 3,085 sires and 33,703 dams). Among the

**Table 1.** Pedigree structure

| Information source | Total no. | Genotyped no. |
|---|---|---|
| Animal from pedigree data | 48,977 | 12,853 |
| Sire from pedigree data | 3,085 | 522 |
| Dam from pedigree data | 33,703 | 134 |
| Cows with phenotypes | 12,331 | 12,331 |
| With genotyped sires | | 11,448 |
| With genotyped dams | | 151 |

sires, 250 sires (202 with genotypes) had 1 to 4 genotyped daughters, 305 sires (274 with genotypes) had 5 to 50 genotyped daughters, 33 sires (31 with genotypes) had 51 to 100 genotyped daughters, and 17 sires (15 with genotypes) had more than 100 genotyped daughters. One genotyped sire had 772 genotyped daughters. Only 134 dams were genotyped. Detailed information for animals with phenotypes and genotypes is given in Table 1.

### Meteorological Data

The weather station nearest to the farm was identified using longitude and latitude information of farms and weather stations and applying the Geosphere package in R (Hijmans et al., 2016). In this regard, 31 different weather stations were allocated to the 56 different farms. The maximum distance between a weather station and a farm was 32.8 km, and the minimum distance was 1.2 km. Hourly THI was calculated based on hourly temperature (T) and hourly relative humidity (RH) as follows (NRC, 1971):

$$\mathrm{THI} = \left(1.8 \times \mathrm{T} + 32\right) - \left(0.55 - 0.0055 \times \mathrm{RH}\right) \times \left(1.8 \times \mathrm{T} - 26\right).$$

The average THI 3 d before the test date was merged with the respective test-day record (Bohmanova et al., 2008).

### Statistical Models

Genomic RRM for MY and SCS considered only the time-dependent covariate DIM (model 1) or DIM and THI simultaneously (model 2). The gRRM (Equation 1) was

$$y_{ijklmn} = \mathrm{HTD}_i + \mathrm{MF}_j + \sum_{o=1}^{q} \alpha_{ko} \mathbf{z}_o\left(d\right) + \sum_{o=1}^{q} \beta_{lo} \mathbf{z}_o\left(d\right) + \sum_{o=1}^{q} \gamma_{mo} \mathbf{z}_o\left(d\right) + e_{ijklmn} \quad [1]$$

and the gRRM (Equation 2) was

$$y_{ijklmn} = \mathrm{HTD}_i + \mathrm{MF}_j + \sum_{o=1}^{q} \alpha_{ko} \mathbf{z}_o\left(d\right) + \sum_{o=1}^{q} \beta_{lo} \mathbf{z}_o\left(d\right) + \sum_{o=1}^{q} \gamma_{mo} \mathbf{z}_o\left(d\right) + \sum_{o=1}^{q} \delta_{lo} \mathbf{z}_o\left(t\right) + \sum_{o=1}^{q} \varepsilon_{mo} \mathbf{z}_o\left(t\right) + e_{ijklmn}, \quad [2]$$

where $y_{ijklmn}$ was the $n$th test-day record for the $m$th cow; $\mathrm{HTD}_i$ was a fixed effect for the $i$th herd test-date, $\mathrm{MF}_j$ was a fixed effect for the $j$th milking frequency (2 or 3 times per day, or milking robot); $\alpha_{ko}$ was the $o$th fixed regression coefficient specific for the $k$th age at first calving class ($k$ = 20 classes; minimum: 20 mo, maximum: 39 mo) within DIM classes (DIM were grouped into 30 classes: 5–15 d, 16–25 d, . . ., 296–305 d); $\beta_{lo}$ and $\delta_{lo}$ were the $o$th random regression coefficients for additive genetic effects for the $l$th animal by DIM classes and by THI classes, respectively (THI was grouped into 13 classes: 35–44, 45–46, 47–48, . . ., 67–68); $\gamma_{mo}$ and $\varepsilon_{mo}$ were the $o$th random regression coefficient for the permanent environmental effects for the $m$th cow by DIM and by THI classes, respectively; $q$ was the number of covariates; $\mathbf{z}_o(d)$ was the vectors of covariates of size $q$ describing the shape of the lactation curve for fixed and random regressions evaluated at the $d$th DIM class; $\mathbf{z}_o(t)$ was the vector of covariates of size $q$ describing the shape of the lactation curve of fixed and random regressions at the $t$th THI class; and $e_{ijklmn}$ was the random residual effect. The classification of DIM and THI values was done to reduce computation time. Fixed and random regressions were modeled using Legendre polynomials of order 2.

The (co)variance structure for model 1 was

$$r\begin{bmatrix}\beta\\ \gamma\\ e\end{bmatrix} = \begin{bmatrix}\mathbf{H}\otimes\mathbf{W}_{\beta} & 0 & 0\\ 0 & \mathbf{I}_m\otimes\mathbf{P}_{\gamma} & 0\\ 0 & 0 & \mathbf{I}_n\sigma_e^2\end{bmatrix}$$

and the (co)variance structure for model 2 was

$$Var\begin{bmatrix}\beta\\ \delta\\ \gamma\\ \varepsilon\\ e\end{bmatrix} = \begin{bmatrix}\mathbf{H}\otimes\mathbf{W}_{\beta} & \mathbf{H}\otimes\mathbf{W}_{\beta\delta} & 0 & 0 & 0\\ \mathbf{H}\otimes\mathbf{W}_{\delta\beta} & \mathbf{H}\otimes\mathbf{W}_{\delta} & 0 & 0 & 0\\ 0 & 0 & \mathbf{I}_m\otimes\mathbf{P}_{\gamma} & \mathbf{I}_m\otimes\mathbf{P}_{\gamma\varepsilon} & 0\\ 0 & 0 & \mathbf{I}_m\otimes\mathbf{P}_{\varepsilon\gamma} & \mathbf{I}_m\otimes\mathbf{P}_{\varepsilon} & 0\\ 0 & 0 & 0 & 0 & \mathbf{I}_n\sigma_e^2\end{bmatrix},$$

where $\mathbf{W}_{\beta}$ and $\mathbf{W}_{\delta}$ were 3 × 3 (co)variance matrices of random regression coefficients for the additive genetic effects by DIM and THI classes, respectively; $\mathbf{W}_{\beta\delta}$ and $\mathbf{W}_{\delta\beta}$ were 3 × 3 covariance matrices for the additive

genetic effects for combinations of DIM and THI classes, respectively; $\mathbf{P}_{\gamma}$ and $\mathbf{P}_{\varepsilon}$ were 3 × 3 (co)variance matrices of random regression coefficients for permanent environmental effects by DIM and THI, respectively; $\mathbf{P}_{\gamma\varepsilon}$ and $\mathbf{P}_{\varepsilon\gamma}$ were 3 × 3 covariance matrices for permanent environmental effects for combinations of DIM and THI classes, respectively; ⊗ denotes the Kronecker product of matrices; $\sigma_e^2$ was the residual variance; and $\mathbf{I}_m$ and $\mathbf{I}_n$ were identity matrices for permanent environmental effects considering $m$ cows and residual effects considering $n$ observations, respectively.

In single-step genomic best linear unbiased prediction models as developed by Aguilar et al. (2010), the matrix $\mathbf{H}$ combines the pedigree-based numerator relationship matrix $\mathbf{A}$ with the genomic relationship matrix $\mathbf{G}$ to consider animals with and without genomic information simultaneously. The inverse of $\mathbf{H}$ was defined as

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & \mathbf{G}^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix}.$$

The $\mathbf{G}$ matrix was constructed according to VanRaden (2008).

In the pRRM, the pedigree-based relationship matrix $\mathbf{A}$ was used instead of the $\mathbf{H}$ matrix. Hence, model 3 was a pRRM in analogy to model 1, just considering the continuous DIM effect. Model 4 was a pRRM in analogy to model 2 (i.e., considering both effects DIM and THI). All analyses were conducted using the GIBBS2F90 program from the BLUPF90 software package (Misztal et al., 2002) using a Bayesian framework via Gibbs sampling. In total, 60,000 samples were generated, and the first 10,000 samples were discarded as burn-in. Posterior means and standard deviations of (co)variance components were calculated from every 50th sample. The number of samples and the length of the burn-in period were determined based on visual inspections for all estimates.

### Estimation of Genetic Parameters

The additive genetic and permanent environmental (co)variances matrices were calculated as $\mathbf{\Phi W \Phi'}$ and $\mathbf{\Phi P \Phi'}$, respectively, where $\mathbf{\Phi}$ was a matrix of Legendre polynomial functions for DIM or THI classes. The elements on the diagonals were additive genetic $\left(\sigma_a^2\right)$ and permanent environmental $\left(\sigma_{pe}^2\right)$ variances for each DIM or THI class. The covariances between the $i$th DIM and $j$th THI classes were calculated as $\mathbf{\Phi}_i \mathbf{W}_{\beta\delta} \mathbf{\Phi'}_j$ and $\mathbf{\Phi}_i \mathbf{P}_{\gamma\varepsilon} \mathbf{\Phi'}_j$ for additive genetic and permanent environmental effects, respectively. As a consequence, the MY or SCS heritability for the $i$th DIM class within the $j$th THI class $\left(h_{(ij)}^2\right)$ was

$$h_{(ij)}^2 = \frac{\sigma_{a\beta(i)}^2 + \sigma_{a\delta(j)}^2 + 2\sigma_{a\beta\delta(ij)}}{\sigma_{a\beta(i)}^2 + \sigma_{a\delta(j)}^2 + 2\sigma_{a\beta\delta(ij)} + \sigma_{p\gamma(i)}^2 + \sigma_{p\varepsilon(j)}^2 + 2\sigma_{p\gamma\varepsilon(ij)} + \sigma_e^2},$$

where $\sigma_{a\beta(i)}^2$ and $\sigma_{a\delta(j)}^2$ were additive genetic variances for MY or SCS from the $i$th DIM and $j$th THI classes, respectively; $\sigma_{p\gamma(i)}^2$ and $\sigma_{p\varepsilon(j)}^2$ were permanent environmental variances for MY or SCS from the $i$th DIM and $j$th THI classes, respectively; $\sigma_{a\beta\delta(ij)}$ and $\sigma_{p\gamma\varepsilon(ij)}$ were additive genetic and permanent environmental covariances for MY or SCS between the $i$th and $j$th classes of DIM and THI, respectively; and $\sigma_e^2$ was the residual variance for MY or SCS. Further model improvement might be due to the consideration of heterogeneous residual variances. However, such modeling implies longer computation time (e.g., Lillehammer et al., 2009). Furthermore, in a comprehensive study by Fujii and Suzuki (2006), modeling of heterogeneous residual variances over years did not affect the ranking of Japanese Holstein sires.

Random regression coefficient solutions were used to estimate genomic breeding values (**GEBV**) for specific DIM and THI classes. The sum of all GEBV for each individual across DIM or THI classes was the total GEBV for the first lactation ($\mathbf{GEBV_{DIM}}$) or for the whole THI range ($\mathbf{GEBV_{THI}}$), respectively. Accordingly, the conventional EBV based on the pedigree relationship matrix for the first whole lactation was $\mathrm{EBV_{DIM}}$, and the EBV for the whole THI range was $\mathrm{EBV_{THI}}$.

### Validation of Genomic and Pedigree-Based Predictions

A 5-fold cross-validation as introduced by Ovenden et al. (2018) was used to compare prediction accuracies from gRRM and pRRM with or without modeling the G×E interactions. For cross-validations, sires were randomly allocated to 5 different groups. Daughter records of sires from 4 groups were used as a training set, and records from remaining daughters from group 5 were considered as a validation set (Figure 1). Daughter group allocations according to the sire information was done to minimize genetic relationships among groups. In the basic runs for all models, GEBV or EBV for all animals were estimated considering the phenotypes from all cows in all groups. In the ongoing run, phenotypes from cows in group 5 (validation set) were discarded. For each model, the prediction accuracy was

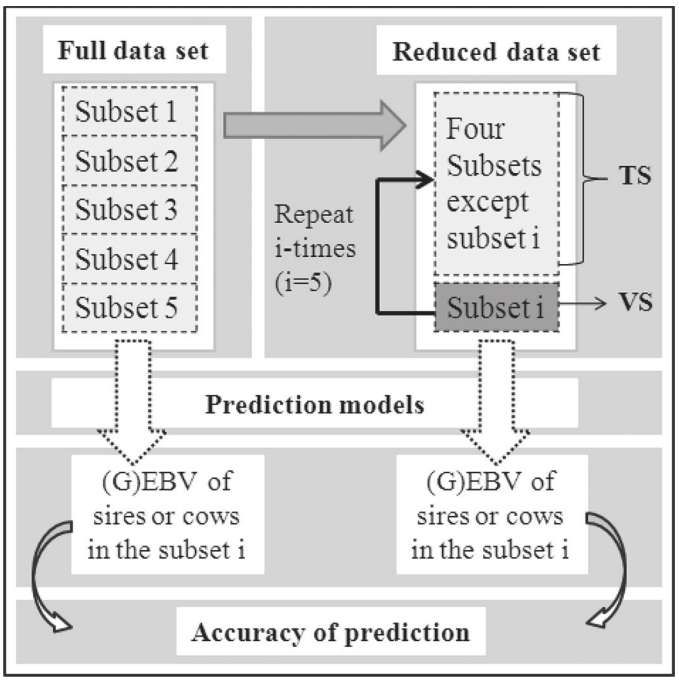


**Figure 1.** Strategy for the creation of training (TS) and validation (VS) sets and the estimation of prediction accuracy. GEBV = genomic breeding value.

the correlation between breeding values for sires and daughters in the validation set from the reduced data (i.e., without cow phenotypes in group 5) with breeding values for corresponding sires and daughters in the full data set (considering the phenotypes from cows in group 5). Finally, the average prediction accuracy from 5 replicates was calculated.

## RESULTS AND DISCUSSION

### Meteorological Data and Traits

Average monthly temperatures, relative humidity, and THI from 2010 to 2015 are shown in Figure 2. Monthly temperatures and THI were lowest in the winter season (December–February) and highest during the summer months (June–August). Over 6 yr, there was a general increase in monthly mean temperature and THI, especially for winter months. Relative humidity was quite constant in the considered time span. In total, 9.94% of test-day records matched with THI values larger than 62, indicating heat stress for dairy cows in Europe (Hammami et al., 2015).

Milk yield was highest (32.87 kg) for the DIM class considering DIM 56 to 65 and lowest (25.82 kg) at the end of lactation for DIM 296 to 305. The maximal SCS (4.24) was identified at the beginning of lactation. The antagonistic relationship between MY and SCS, especially in early lactation, is in line with studies by, for example, de los Campos et al. (2006) or Jamrozik et al. (2010). For all lactation stages, MY was quite constant until THI 62. Afterward, for larger THI, MY decreased significantly. Test-day SCS continuously increased with increasing THI, especially in the early-lactation period. Results correspond with previous evaluations of THI effects on production traits in middle Europe (Hammami et al., 2013, 2015).

### Genetic Parameters

***Models Without G×E Interactions.*** Heritabilities from model 1 and model 3 for specific DIM classes are shown in Figure 3. As expected, heritabilities for MY were larger than heritabilities for SCS. Heritabilities for MY from model 1 were lowest in the early lactation stage and increased gradually to 0.35 at the end of lactation. Generally, for MY, heritabilities from the pedigree-based model 3 (0.20 to 0.46 from early to late lactation, respectively) were larger than heritabilities (0.19 to 0.35 from early to late lactation, respectively) from the genomic model 1. Accordingly, inflated heritabilities at the peripheries of lactations were reported in previous pRRM applications (Strabel et al., 2005; Zavadilová et al., 2005). Heritabilities for SCS were quite constant throughout lactation and ranged between 0.07 and 0.09 from model 1 and between 0.08 and 0.09 from model 3. Heritabilities by DIM from the pRRM without G×E interaction effect (i.e., model 3) were in line with estimates from previous studies (Zavadilová et al., 2005; Nishiura et al., 2015). Posterior standard deviation from model 1 (<0.01 for both traits across DIM) were smaller than from model 3 (range from 0.01 to 0.04 for both traits across DIM), indicating a larger accuracy for variance component estimations when considering the genomic relationship matrix (Bérénos et al., 2014).

***Models with G×E Interactions.*** Model 2 and model 4 allowed the estimation of (co)variance components for all combinations of THI × THI and THI × DIM classes. Genetic parameters from models 2 and 4 for MY and for SCS are presented in Figures 4 and 5, respectively, for the different class combinations. Additive genetic variances for both traits MY (Figure 4A) and SCS (Figure 5A) varied across different combinations of DIM × THI classes. Hence, additive genetic variances depended on the environmental gradient (i.e., THI) as well as on time alterations (i.e., DIM). Using model 2, the largest additive genetic variance for MY was estimated for the DIM class latest in lactation (296–305 d) combined with the lowest THI class (THI >45). In contrast, for SCS and model 2, the largest additive genetic variance was identified for the last DIM

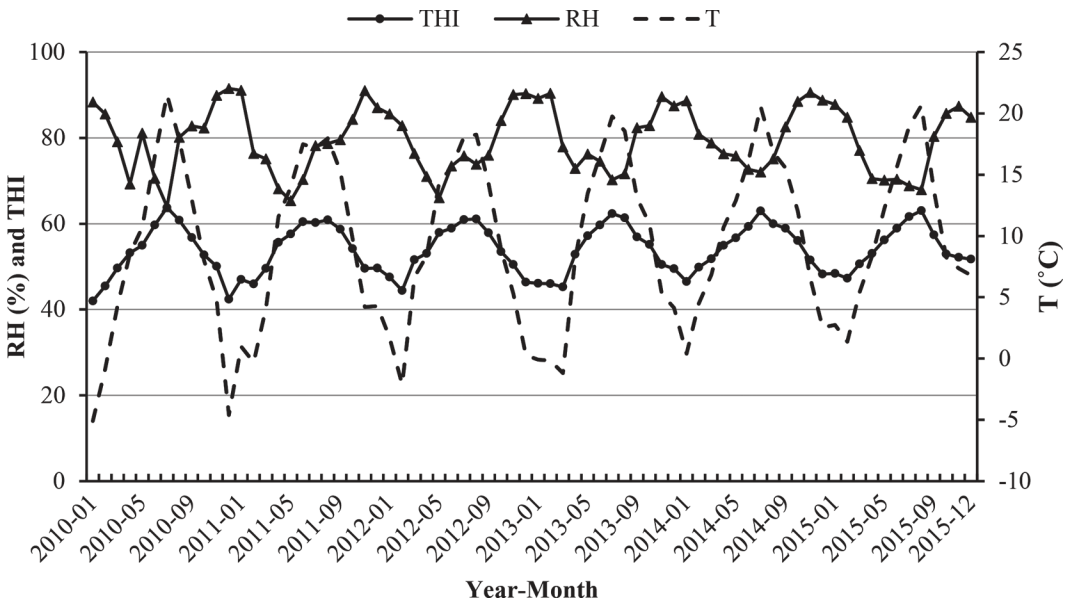


**Figure 2.** The monthly average temperature-humidity index (THI), relative humidity (RH), and temperature (T) from 2010 to 2015.

class combined with the highest THI class (THI 67–68). Hence, we postulate that dairy cows express their genetic potential differently for a production trait (MY) and for a low-heritability functional trait (SCS) under heat stress conditions. Similarly, in such context, Schierenbeck et al. (2011) analyzed daughter yield deviations and identified a better genetic differentiation for SCS in challenging environments. In contrast, for MY, superior management, husbandry, and feeding conditions in large-scale herds contributed to a broader range of yield deviations and to more extreme sire breeding values (Schierenbeck et al., 2011). Accordingly, König et al. (2005) found an increase of additive-genetic variances for protein yield with increasing herd size. They stated, "A reason for this could be that within herd correlations of genotype × management (e.g., as arising from feeding according to the genetic potential) are higher in large farms since all available management tools can be applied which would not be feasible on a small farm."

Permanent environmental variances were largest in the late-lactation stage combined with the highest THI class (i.e., THI 67–68) for both traits MY (Figure 4B) and SCS (Figure 5B). Very similar permanent environmental variances were estimated from models 2 and 4.

For the genomic and pedigree-based models, heritabilities for MY increased by DIM and decreased by THI (Figure 4C). Zwald et al. (2003) focused for herd grouping on several environmental descriptors across country borders. In agreement with results from our study, heritabilities for MY based on data from herds located in hot climates were smaller than heritabilities from herds located in temperate climates. For Brazilian Holstein dairy cattle, Santana et al. (2017) reported the largest production trait heritabilities for low THI values in late lactation. However, they identified opposite heritability trends for SCS. In our study, SCS heritabilities from model 2 and 4 increased with increasing THI at the beginning of lactation but decreased in the late lactation stage (Figure 5C). In the studies by Brügemann et al. (2011) and Bohlouli et al. (2013), protein yield and MY heritabilities slightly altered across THI. Differences in additive genetic variances and heritabilities indicate variation in selection response, additionally depending on the function used for modeling both descriptors DIM and THI. Heritabilities for production traits as a function of THI are different depending on populations and locations (e.g., Ravagnolo and Misztal, 2000; Hammami et al., 2015). Santana et al. (2017) expected the largest selection response for MY in the thermal comfort zone because of the largest MY heritabilities for THI <45. In our study, heritabilities for SCS increased with increasing THI. Accuracy of selection depends on trait heritabilities. Hence, selection response for SCS might increase when considering trait recording in challenging environments (i.e., environments representing heat stress).

When comparing models with or without G×E interactions, heritabilities were larger from models considering the THI component. Accordingly, Abdullahpour et al. (2013) reported significantly larger heritabilities from models considering detailed environmental herd information. In simulations neglecting the G×E interaction, the residual variance component substantially increased, with effect on heritability decreased

(Bohlouli et al., 2013). König et al. (2005) identified smaller permanent and residual variance components in small-scale farms than in large-scale farms. This might be due to the heterogeneous environmental effects in large-scale farms, mainly due to specific feeding and management strategies in created subgroups of cows within herds. Inclusion of the source of variation as a G×E interaction component into statistical modeling might increase the additive genetic variance for the trait of interest while simultaneously decreasing the residual effect (Bowman, 1972).

Smaller heritabilities were estimated when using a gRRM (models 1 and 2) instead of a pRRM (models 3 and 4). For a chicken population, Momen et al. (2017) also reported smaller heritabilities for body measurement and productivity when modeling a genomic relationship matrix. Veerkamp et al. (2011) estimated heritabilities for MY, DMI, and BW of Holstein dairy cattle. Estimates were smaller when considering the genomic relationships. Different (co)variance components from pRRM and gRRM can be expected because for genotyped animals, identity by state relationships and undefined base population structure is used instead of identity by descent relationships (Powell et al., 2010). Snelling et al. (2009) reported that different SNP densities as used for the construction of **G** matrices might contribute to differences in variance component estimates. Moreover, some of the environmental effects might be confounded with pedigree relationships (Lee et al., 2010; Veerkamp et al., 2011). The given arguments might explain heritability differences from the pRRM and the gRRM.

Figure 4D (for MY) and Figure 5D (for SCS) display genetic correlations between all combinations of DIM × THI classes from models 2 and 4. For model 2 and MY, the genetic correlation was smallest (0.26) between DIM 5 to 15 and THI <45, indicating differences in genetic mechanisms for early lactation and for low THI. Early lactation with a natural energy deficiency in high-yielding cows is associated with physiological stress, but THI <45 implies well-being for cows. Accordingly, substantial re-rankings of sires according to GEBV for MY were observed; sires with high GEBV early in lactation had low GEBV for THI <45 and vice versa (Figure 6). The same tendencies were found in previous studies using pedigree-based relationship matrices to depict DIM × THI interactions (e.g., Bohlouli et al., 2013; Hammami et al., 2015). Large genetic correlations (>0.80) between MY from the middle of lactation with MY from all THI classes suggest selection of sires with high genetic values at DIM 100 to 200, to use correlated selection response for heat tolerance. For SCS, the lowest genetic correlation (0.78) was estimated between the latest DIM and the highest THI class (Figure 5D). Hence, there was no evidence for the existence of a significant lactation period

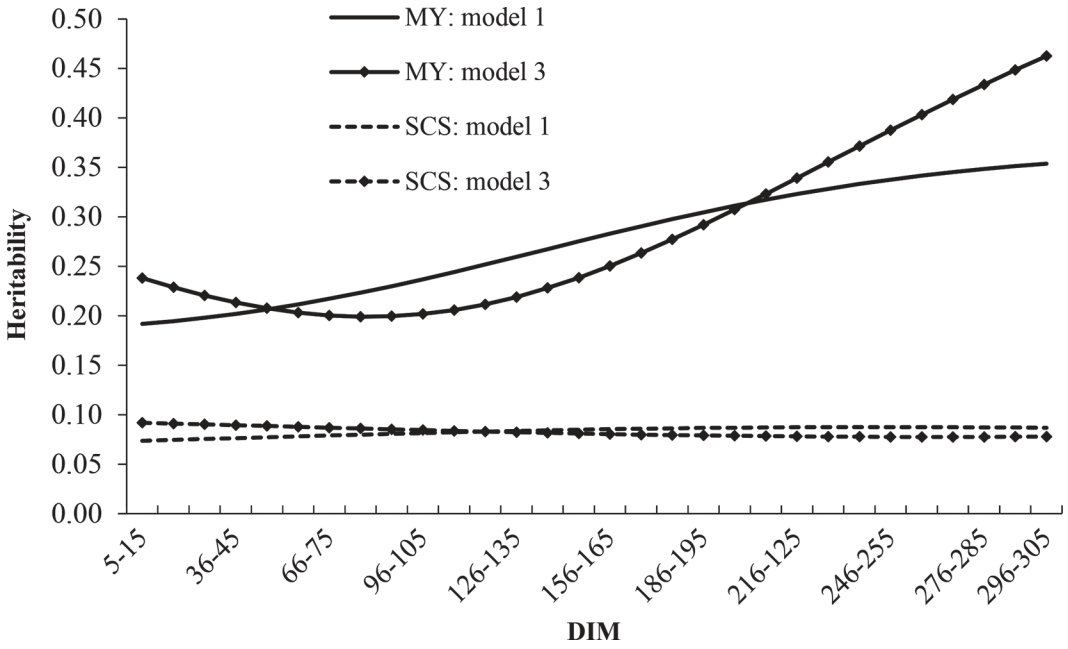


**Figure 3.** Heritability estimates for milk yield (MY) and SCS for different DIM classes estimated with the genomic random regression model 1 and the pedigree-based random regression model 3 (both models without genotype by environment interactions). For MY, posterior SD of heritabilities ranged from 0.011 to 0.014 for model 1 and from 0.027 to 0.041 for model 3. For SCS, posterior SD of heritabilities ranged from 0.007 to 0.008 for model 1 and from 0.011 to 0.014 for model 3.

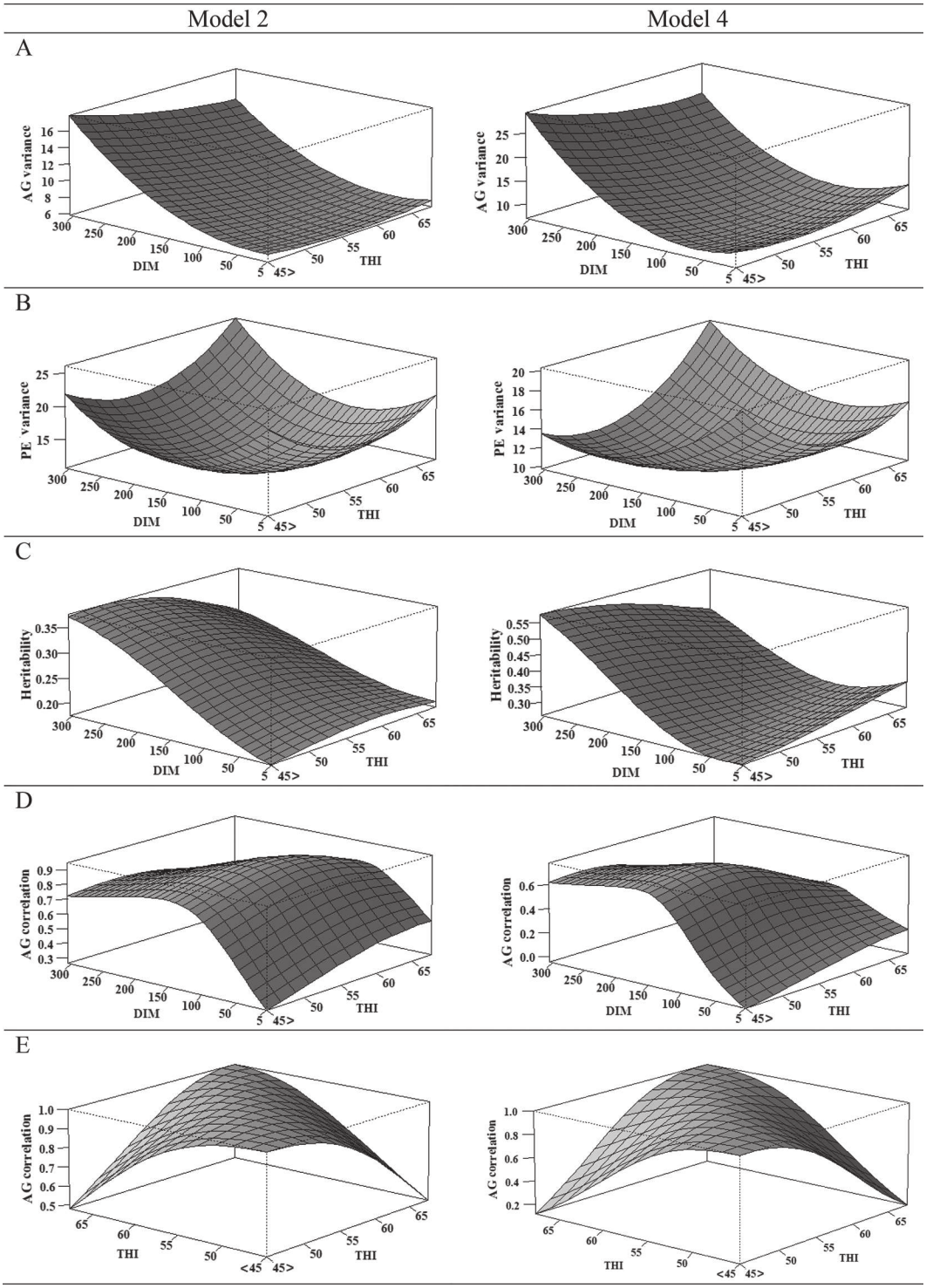


**Figure 4.** Additive genetic (AG) and permanent environmental (PE) variances, heritabilities, and genetic correlations for milk yield (MY) estimated with the genomic random regression model 2 and the pedigree-based random regression model 4 (both models considering genotype by environment interactions) for DIM and temperature-humidity index (THI) combinations. Posterior SD for estimates from model 2 ranged from 0.32 to 0.95 (A), 0.20 to 0.71 (B), 0.01 to 0.02 (C), 0.03 to 0.08 (D), and 0.00 to 0.07 (E). Posterior SD for estimates from model 4 ranged from 0.68 to 2.62 (A), 0.53 to 1.86 (B), 0.02 to 0.04 (C), 0.06 to 0.16 (D), and 0.00 to 0.13 (E).

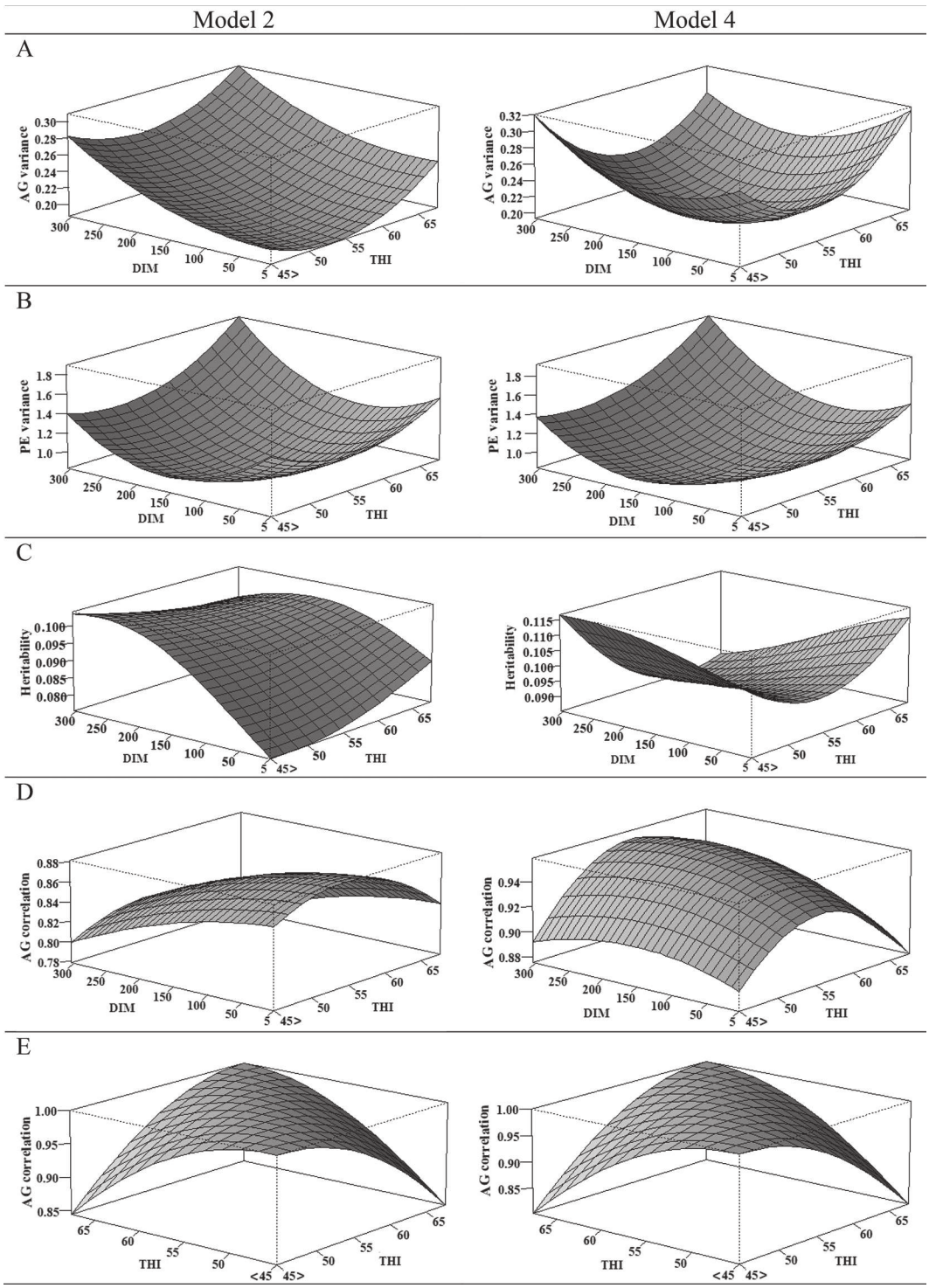


**Figure 5.** Additive genetic (AG) and permanent environmental (PE) variances, heritabilities, and genetic correlations for SCS estimated with the genomic random regression model 2 and the pedigree-based random regression model 4 (both models considering genotype by environment interactions) for DIM and temperature-humidity index (THI) combinations. Posterior SD for estimates from model 2 ranged from 0.02 to 0.03 (A), 0.02 to 0.06 (B), 0.00 to 0.01 (C), 0.05 to 0.09 (D), and 0.00 to 0.09 (E). Posterior SD for estimates from model 4 ranged from 0.02 to 0.07 (A), 0.02 to 0.08 (B), 0.01 to 0.02 (C), 0.04 to 0.11 (D), and 0.00 to 0.12 (E).

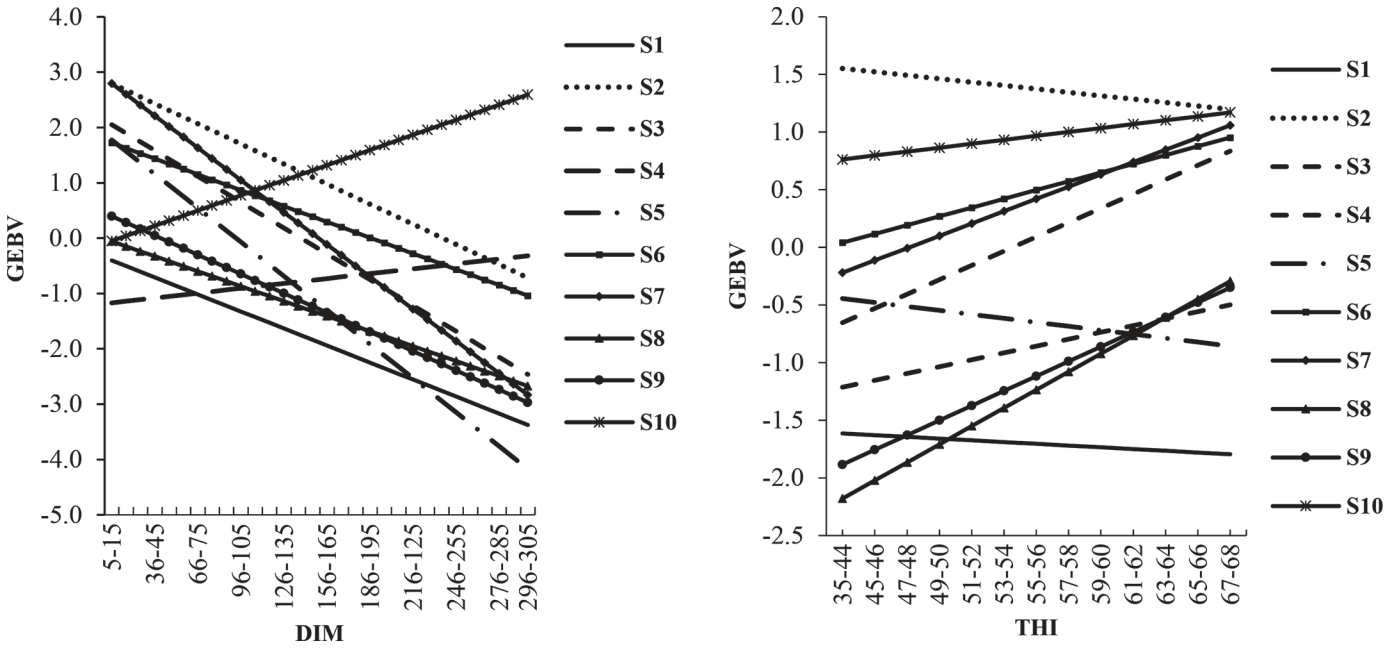


**Figure 6.** Genomic estimated breeding values (GEBV) for milk yield for 10 genotyped sires (S1–S10) with at least 150 genotyped daughters by DIM and temperature-humidity index (THI) classes.

× heat stress interaction for SCS. Accordingly, only slight re-rankings of sires according to GEBV across DIM and THI classes (Figure 7) were observed. Similar results were reported from pedigree-based models, aiming on the exploration of environmental sensitivity of SCS. For instance, genetic correlations for SCS were quite large (>0.80) when grouping herds according to herd parameters [e.g., intraherd standard deviations for milk yield (Castillo-Juarez et al., 2000; Raffrenato et al., 2003) or average herd SCS (Banos and Shook, 1990; Calus et al., 2005)]. However, Calus et al. (2006) found considerable re-rankings of sires according to their SCS

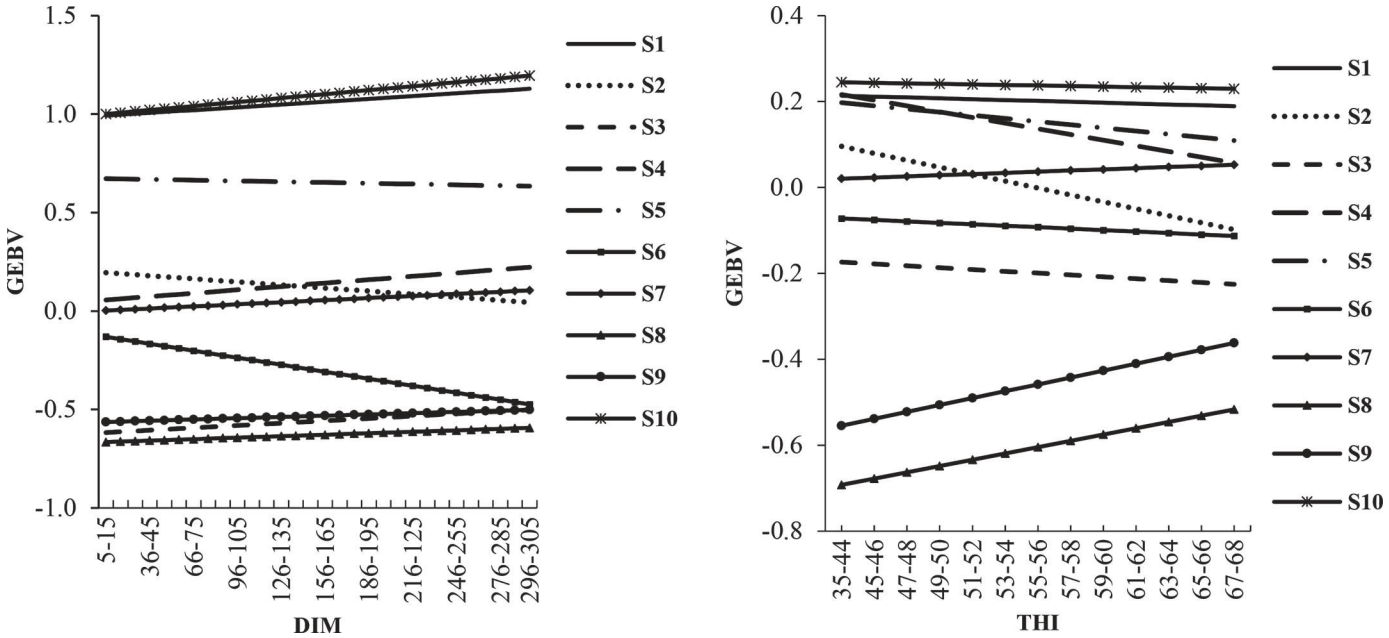


**Figure 7.** Genomic estimated breeding values (GEBV) for SCS for 10 genotyped sires (S1–S10) with at least 150 genotyped daughters by DIM and temperature-humidity index (THI) classes.

EBV for different combinations of bulk milk SCC levels and DIM.

Genetic correlations between the same traits recorded in different THI classes (i.e., model 2 and model 4) decreased with increasing distances between THI classes (Figure 4E for MY; Figure 5E for SCS). Genetic correlations were smallest between MY at THI <45 with MY at THI 67 to 68 (i.e., 0.49 from model 2 and 0.18 from model 4). Genetic correlations for SCS across different THI classes were larger than estimates for MY. The lowest genetic correlation was 0.85 from model 2 and 0.80 from model 4 (Figure 5E). Quite large genetic correlations for SCS across THI classes support the explanations given for the large SCS correlations across DIM × THI combinations (i.e., less environmental sensitivity for SCS compared with MY). Hence, in agreement with Hayes et al. (2016), we suggest consideration of MY from different THI classes as correlated traits in genetic evaluations for German Holsteins.

## Prediction Accuracies

***Models Without G×E Interactions.*** Prediction accuracies from the 5-fold cross-validations are listed in Table 2. In general, prediction accuracies were larger for MY compared with SCS. The heritability, a major parameter for the genetic trait architecture, had substantial effect on prediction accuracies (Goddard and Hayes, 2009; Hayes et al., 2009). Given the same size of a reference population, prediction accuracies increased with increasing trait heritabilities (Goddard and Hayes, 2009; Guo et al., 2014; Yao et al., 2017).

In agreement with Yin et al. (2014) and Forneris et al. (2016), replacing **A** by **G** contributed to an increase of genomic predictions. In our study, consideration of genomic information had substantial effect on prediction accuracies for daughters (i.e., almost a doubling of prediction accuracies). For MY, the prediction accuracy for $GEBV_{DIM}$ of sires via model 1 was 0.82, but the prediction accuracy for the same sires for the conventional EBV ($EBV_{DIM}$) from model 3 was only 0.72. For their daughters, the prediction accuracy was 0.79 from model 1 and 0.39 from model 3. Prediction accuracies were lower for SCS than for MY but also increased for SCS when considering genomic information. A significant prediction accuracy increase when considering genomic information was reported for low-heritability traits (Buch et al., 2012).

***Models with G×E Interactions.*** An increase in prediction accuracies was due to the inclusion of the THI component into statistical modeling and when basing predictions on DIM × THI combinations (Table 2). For only SCS and the pRRM applications, prediction accuracies were slightly larger from model 3 (0.43 for

**Table 2.** Prediction accuracies (SD in parentheses) from the 5-fold cross-validation for sires and their daughters for milk yield (MY) and SCS breeding values for the whole lactation (DIM), the aggregated temperature-humidity index (THI), and the DIM × THI combination (models 1 and 3: without genotype by environment interactions; models 2 and 4: with genotype by environment interactions)

| | | Genomic random regression model | | | | Pedigree-based random regression model | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Model 1 | Model 2 | | | Model 3 | Model 4 | | |
| Trait | Validation | DIM | DIM | THI | DIM-THI | DIM | DIM | THI | DIM-THI |
| MY | Sires | 0.82 (0.042) | 0.89 (0.021) | 0.88 (0.029) | 0.89 (0.023) | 0.72 (0.091) | 0.56 (0.055) | 0.54 (0.092) | 0.85 (0.106) |
| | Daughters | 0.79 (0.026) | 0.83 (0.026) | 0.83 (0.034) | 0.83 (0.029) | 0.39 (0.089) | 0.36 (0.077) | 0.33 (0.079) | 0.37 (0.095) |
| SCS | Sires | 0.76 (0.018) | 0.75 (0.051) | 0.65 (0.084) | 0.81 (0.009) | 0.64 (0.044) | 0.42 (0.094) | 0.43 (0.167) | 0.61 (0.206) |
| | Daughters | 0.72 (0.035) | 0.70 (0.020) | 0.59 (0.060) | 0.75 (0.058) | 0.43 (0.144) | 0.39 (0.043) | 0.41 (0.089) | 0.39 (0.141) |

**Table 3.** Prediction accuracies (SD in parentheses) from the 5-fold cross-validation for sires and their daughters for milk yield (MY) and SCS breeding values for specific DIM and temperature-humidity index (THI) classes, estimated with genomic random regression models

| | | DIM | | | THI | |
|---|---|---|---|---|---|---|
| Trait | Validation | 5–15 | 146–155 | 296–305 | <45 | 67–68 |
| MY | Sires | 0.81 (0.034) | 0.88 (0.021) | 0.88 (0.022) | 0.89 (0.030) | 0.85 (0.016) |
| | Daughters | 0.77 (0.045) | 0.83 (0.026) | 0.81 (0.027) | 0.83 (0.025) | 0.80 (0.039) |
| SCS | Sires | 0.73 (0.044) | 0.75 (0.051) | 0.76 (0.057) | 0.65 (0.113) | 0.71 (0.106) |
| | Daughters | 0.67 (0.047) | 0.69 (0.022) | 0.70 (0.017) | 0.59 (0.066) | 0.60 (0.062) |

daughters, 0.64 for sires) than for the combined DIM × THI effect from model 4 (0.39 for daughters, 0.61 for sires). Somatic cell score seems to be a specific trait in genomic and genetic evaluations for German Holsteins. Despite low heritabilities, SCS is the trait with highest accuracies for GEBV. Yin and König (2018) related genetic parameters to genomic herd descriptors. Also in this study, the genetic (co)variance pattern for SCS differed from those for milk or protein yield.

Regarding the gRRM considering THI (model 2), MY prediction accuracies for $GEBV_{THI}$ were 0.88 for sires and 0.83 for their daughters. A slight prediction accuracy increase was due to the GEBV construction considering genomic effects for DIM × THI classes simultaneously. The prediction accuracy for $GEBV_{DIM\text{-}THI}$ was 0.89 for sires (plus 0.07 compared with model 1) and 0.83 for their daughters (plus 0.04 compared with model 1). For SCS, prediction accuracies for $GEBV_{THI}$ were lower than for $GEBV_{DIM}$ (0.65 vs. 0.75 for sires; 0.59 vs. 0.70 for daughters). Regarding genomic models and SCS, prediction accuracies were largest for $GEBV_{DIM\text{-}THI}$. The gain in prediction accuracy when calculating $GEBV_{DIM\text{-}THI}$ instead of $GEBV_{DIM}$ or instead of $GEBV_{THI}$ was larger for SCS compared with MY.

The accuracies of genomic predictions for selected DIM (i.e., prediction accuracies for $GEBV_{DIM}$) and for specific THI (i.e., prediction accuracies for $GEBV_{THI}$) are given in Table 3. For both traits MY and SCS, accuracies were larger for the middle and the end of lactation compared with the early-lactation period. In contrast to MY, prediction accuracies for SCS were larger under heat stress (THI 67–68) compared with THI <45, especially for sires. Generally, prediction accuracies of selected DIM × THI combinations (i.e., prediction accuracies for $GEBV_{DIM\text{-}THI}$; Table 4) followed the same pattern for results as given in Table 3. For MY, prediction accuracies were throughout larger for THI <45, but for SCS and genomic prediction of sires, a light prediction accuracy increase under heat stress conditions (THI 67–68) was observed.

Genomic RRM with a THI gradient allowed the estimation of $GEBV_{THI}$ and $GEBV_{DIM\text{-}THI}$. As a further advantage, prediction accuracies for $GEBV_{DIM}$ increased when modeling the THI effect (model 2) compared with a gRRM considering only the time-dependent covariate DIM (model 1). Both types of RRM (i.e., gRRM and pRRM) consider longitudinal data as correlated traits. Information from genetically correlated traits in multitrait genomic models improved genomic predictions over single-trait genomic predictions (Guo et al., 2014; Jiang et al., 2015). In simulations, further increase in prediction accuracy was due to consideration of G×E interaction terms for gRRM (Yin et al., 2014) as well as for multitrait genomic model applications (Bohlouli et al., 2017). As a further advantage, such modeling allows detection of QTL expressions in specific environments (Lillehammer et al., 2007). Tiezzi et al. (2017) considered different environmental descriptors (e.g., herd management, latitude and altitude, geographical region, herd fertility, and meteorological effects) in models with G×E interactions. However, apart from meteorological effects, such G×E modeling only marginally improved genomic prediction accuracies for genotyped bulls. Hence, climate information seems to

**Table 4.** Prediction accuracies (SD in parentheses) from the 5-fold cross-validation for sires and their daughters for milk yield (MY) and SCS breeding values for combinations of specific DIM and temperature-humidity index (DIM × THI) classes, estimated with genomic random regression models

| | | THI <45 | | | THI 67–68 | | |
|---|---|---|---|---|---|---|---|
| Trait | Validation | 5–15 DIM | 146–155 DIM | 296–305 DIM | 5–15 DIM | 146–155 DIM | 296–305 DIM |
| MY | Sires | 0.86 (0.006) | 0.89 (0.026) | 0.89 (0.027) | 0.82 (0.026) | 0.87 (0.017) | 0.88 (0.021) |
| | Daughters | 0.82 (0.017) | 0.84 (0.024) | 0.83 (0.025) | 0.79 (0.035) | 0.82 (0.035) | 0.82 (0.026) |
| SCS | Sires | 0.76 (0.012) | 0.80 (0.021) | 0.80 (0.028) | 0.77 (0.031) | 0.81 (0.019) | 0.81 (0.013) |
| | Daughters | 0.71 (0.072) | 0.74 (0.046) | 0.76 (0.031) | 0.69 (0.119) | 0.72 (0.088) | 0.75 (0.062) |

be a crucial environmental parameter to improve the accuracy of breeding values in genomic evaluations. For both traits, utilization of model 4 instead of model 3 did not improve prediction accuracies. Hence, we suggest consideration of a larger number of phenotypic records to increase prediction accuracies in pRRM with interaction terms (Meseret et al., 2015). An increase of both data sources, phenotypic performances and genomic information for specific DIM and THI classes, allows a target-orientated selection in commercial dairy cattle herds in the context of precision farming. According to Bewley et al. (2015), precision farming contributes to the improvement of cow management strategies to optimize farm economy via utilization of modern technologies. Genetic marker technology allows phenotype prediction of genotyped female calves or heifers (Yin and König, 2016), which can be optimized when considering environmental particularities via G×E interaction models. An increase of SNP markers and test-day records per genotyped animals for a large number of THI classes and the application of G×E interaction models will gradually contribute to improvements of genomic predictions.

## CONCLUSIONS

Genomic RRM considering G×E interactions contributed to an increase of prediction accuracies of breeding values for sires and their daughters. Hence, the availability of a large data set including genotyped cows with longitudinal test-day records for a broad THI range allows genomic predictions for extreme THI classes representing limited phenotypic data. In addition, for genomic models with G×E interactions, posterior standard deviations of genetic (co)variance components were very small. Genetic parameters and prediction accuracies from the gRRM were more accurate than those from the pRRM. As a further disadvantage for the pRRM, heritabilities from the extreme THI classes were inflated. Regarding gRRM applications, some genetic correlations for MY and specific THI × THI or THI × DIM combinations were extremely small (e.g., only 0.26 between MY recorded at DIM 5–15 and MY recorded at THI <45). Hence, it is imperative to consider G×E interactions via THI gradients in gRRM for MY. Such a modeling strategy for MY (i.e., simultaneous consideration of DIM and THI) also improved prediction accuracies of GEBV for specific DIM compared with a simpler model just considering the DIM covariate. Nevertheless, regarding practical implementations, we found increasing computation time for a gRRM accounting for G×E interactions (26 d compared with a genomic model without G×E interactions).

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## 2.3. Original research paper 3

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# Simulation, prediction, and genetic analyses of daily methane emissions in dairy cattle

**T. Yin,*† T. Pinent,* K. Brügemann,* H. Simianer,† and S. König*[1]**
*Department of Animal Breeding, University of Kassel, 37213 Witzenhausen, Germany
†Department of Animal Science, Animal Breeding and Genetics Group, Georg-August-Universität Göttingen, 37075 Göttingen, Germany

## ABSTRACT

This study presents an approach combining phenotypes from novel traits, deterministic equations from cattle nutrition, and stochastic simulation techniques from animal breeding to generate test-day methane emissions (MEm) of dairy cows. Data included test-day production traits (milk yield, fat percentage, protein percentage, milk urea nitrogen), conformation traits (wither height, hip width, body condition score), female fertility traits (days open, calving interval, stillbirth), and health traits (clinical mastitis) from 961 first lactation Brown Swiss cows kept on 41 low-input farms in Switzerland. Test-day MEm were predicted based on the traits from the current data set and 2 deterministic prediction equations, resulting in the traits labeled MEm1 and MEm2. Stochastic simulations were used to assign individual concentrate intake in dependency of farm-type specifications (requirement when calculating MEm2). Genetic parameters for MEm1 and MEm2 were estimated using random regression models. Predicted MEm had moderate heritabilities over lactation and ranged from 0.15 to 0.37, with highest heritabilities around DIM 100. Genetic correlations between MEm1 and MEm2 ranged between 0.91 and 0.94. Antagonistic genetic correlations in the range from 0.70 to 0.92 were found for the associations between MEm2 and milk yield. Genetic correlations between MEm with days open and with calving interval increased from 0.10 at the beginning to 0.90 at the end of lactation. Genetic relationships between MEm2 and stillbirth were negative (0 to −0.24) from the beginning to the peak phase of lactation. Positive genetic relationships in the range from 0.02 to 0.49 were found between MEm2 with clinical mastitis. Interpretation of genetic (co)variance components should also consider the limitations when using data generated by prediction equations. Prediction functions only describe that part of MEm which is dependent on the factors and effects included in the function. With high probability, there are more important effects contributing to variations of MEm that are not explained or are independent from these functions. Furthermore, autocorrelations exist between indicator traits and predicted MEm. Nevertheless, this integrative approach, combining information from dairy cattle nutrition with dairy cattle genetics, generated novel traits which are difficult to record on a large scale. The simulated data basis for MEm was used to determine the size of a cow calibration group for genomic selection. A calibration group including 2,581 cows with MEm phenotypes was competitive with conventional breeding strategies.

**Key words:** predicted methane emissions, genetic parameters, random regression models

## INTRODUCTION

Modern dairy cattle breeding goals incorporate a variety of traits representing the overall categories of productivity and functionality. Breeding goals will continue to be extended by the direct inclusion of additional functional traits mainly reflecting health and product quality (Boichard and Brochard, 2012). In addition, and especially when following the consumers' perspective, dairy cattle's environmental impact or resource efficiency will play a major role in future breeding strategies (König et al., 2013).

As a by-product of bacterial fermentation in ruminants, greenhouse gas (**GHG**) emissions, mainly including $CH_4$, contribute to global climate change and an inefficient use of dietary energy. The dairy cattle sector accounts for 4% of the total global anthropogenic GHG emissions, with a 52% contribution from methane (FAO, 2010). Controlling and mitigating of methane emissions (**MEm**) is imperative because the expected global warming potential for MEm is 25 times larger than for $CO_2$ (Forster et al., 2007). Several methods to measure enteric MEm from ruminants can be applied, whereas the most traditional and accurate method is

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[1]Corresponding author: sven.koenig@uni-kassel.de

the use of respiration chambers (Muñoz et al., 2012). This expensive method requires tremendous logistical efforts, and can only be applied to a limited number of individuals. The sulfur hexafluoride tracer technique (Johnson et al., 1994) was used to measure MEm from individuals kept under grazing conditions. However, when using this tracer technique, a permeation tube containing sulfur hexafluoride has to be placed into the cow's rumen and a sampling apparatus must be attached to the cow. Moreover, this method does not allow measuring the small amount of MEm produced in the large intestine (Murray et al., 1976). A further indicator used for the prediction of individual MEm is based on samples from milk (i.e., FA compositions measured by GC; e.g., Chilliard et al., 2009) or based on milk mid-infrared spectral data (Dehareng et al., 2012). This method requires access to milk laboratories with capacities for analyzing and saving spectral data as well as the development and validation of prediction equations. Utilization of a mobile laser methane detector allows direct on-farm measurements of breath MEm without disturbing the natural behavior of cows (Chagunda et al., 2009). However, high costs for the mobile equipment including technician input, hamper commercial application. The same applies to measurements of breath MEm of individual cows by using the Fourier transform infrared method (Lassen et al., 2012).

A variety of animal-associated and environmental effects contribute to variations of daily MEm. Cow-specific effects include milk productivity (Garnsworthy, et al., 2012a), parity, BW, and stage of lactation (Bell, et al., 2011; Garnsworthy, et al., 2012b). Major environmental factors reflect influences of feeding systems and of feeding strategies (Vlaming et al., 2005). Feeding components include variations of MEm due to diet compositions (Yan et al., 2006) and due to the amount of fluids in diets and further nutritional factors (Hegarty and McEwan, 2010). Also, the recording technique used affects the accuracy of MEm measurements (Muñoz et al., 2012). Direct MEm measurements are associated with technical challenges and high costs, implying the development of MEm prediction equations. Available prediction equations are based on information from a limited number of cows kept in experimental herds and on data from feed rations combined with physiological parameters (e.g., Kirchgessner et al., 1995; Haas et al., 2011; Garnsworthy et al., 2012b). Furthermore, prediction equations build upon different assumptions (e.g., with regard to predefined levels of energy required for maintenance and for productivity). Nevertheless, considerable MEm variation was detected also for dairy cows fed the same diet (Grainger et al., 2007) and housed under identical commercial conditions (Garnsworthy et al., 2012b). Substantial MEm variation in spite of identical environmental conditions indicates differences on the genetic scale. A heritable component for MEm is a prerequisite for implementing sustainable breeding strategies to reduce GHG and to improve resource efficiency of dairy cattle farming.

Moderate heritabilities in the range of 0.30 to 0.35 for predicted and real measurements of MEm were reported for dairy cows and sheep (Haas et al., 2011; Pinares-Patiño et al., 2011). Positive genetic correlations were found between predicted MEm and fat- and protein-corrected milk yield (0.31), as well as between MEm and residual feed intake (0.31; Haas et al., 2011). Such moderate genetic relationships suggest the use of MEm as an indicator for feed efficiency. Inclusion of MEm into overall breeding goals requires additional genetic covariances and genetic correlations between MEm with fertility and with health traits. Consequently, the objectives of the present study were (1) to develop a strategy which combines deterministic equations and stochastic simulations to predict daily MEm based on routinely recorded on-farm data; (2) to estimate daily heritabilities and genetic variances for predicted longitudinal MEm with random regression models; (3) to estimate genetic correlations between predicted longitudinal MEm with test-day production traits [milk yield (**MY**), fat percentage (**Fat%**), protein percentage (**Pro%**) and MUN], fertility traits [calving interval (**CI**), days open (**DO**), and stillbirth (**SB**)], and with the binary health trait clinical mastitis (**CM**); and (4) to evaluate a variety of direct and indirect MEm breeding strategies with and without genomic information.

## MATERIALS AND METHODS

### *Data*

Basis for data generation and data analyses were 916 first parity Brown Swiss cows born between 2000 and 2007. The cows were kept on 41 low-input farms located in mountainous regions of Switzerland. Herd size ranged from 9 to 49 cows, with an average of 22.34 cows per herd. The average number of observations per contemporary group (herd × test-year-season) included 7.05 cows. A total of 911 cows were daughters of 274 sires (5 cows had unknown parents), indicating an average of 3.32 daughters per sire. The genetic structure was as follows: 138 sires had only 1 daughter, 105 sires had 2 to 5 daughters, 13 sires had 6 to 10 daughters, 10 sires had 11 to 20 daughters, 6 sires had 21 to 30

daughters, and 3 sires had 31 to 50 daughters. The largest progeny include 47 daughters per sire.

Test-day production traits were repeated measurements for MY, Fat%, Pro%, and MUN with a minimum of 2 and a maximum of 8 observations per cow. Conformation traits included wither height (**WH**), hip width (**HW**), and BCS. Reproduction traits of interest were the continuously distributed traits CI and DO and the binary trait SB. Following Yin et al. (2014) for health data preparation, CM was defined as an all-or-none binary trait with 1 representing occurrence of CM within −1 d before to 120 d after calving, and 0 representing healthy cows. Body weight of cows was predicted based on formula [1], developed by Enevoldsen and Kristensen (1997):

$$\begin{aligned}\text{BW} = 439 + 0.2 \times \text{DIM} + 4.2 \times \text{HH} + 29.2 \times \text{HW} \\ + 0.3 \times \text{HW}^2 + 33.5 \times \text{BCS}.\end{aligned} \quad [1]$$

Hip height (**HH**) was not considered in the official type trait classification system for Brown Swiss cows until August 2010. Due to the close correlation between HH and WH (London et al., 2012), we replaced HH with WH in equation [1]. Daily ECM was calculated using formula [2], as introduced by Haas et al. (2011):

$$\begin{aligned}\text{ECM} = (0.337 + 0.116 \times \text{Fat\%} + 0.06 \\ \times \text{Pro\%}) \times \text{MY}.\end{aligned} \quad [2]$$

### *Prediction of Daily MEm*

Two different equations were applied to predict daily MEm for the first-parity cows from the low-input production systems, resulting in MEm1 and MEm2. Equation [3], used for the calculation of MEm1, was introduced by Kirchgessner et al. (1995) and is based only on records for MY and metabolic BW ($\text{BW}^{0.75}$):

$$\begin{aligned}\text{MEm1} = (10.0 + 4.9 \times \text{MY} + 1.5 \times \text{BW}^{0.75}) \\ \times 0.0132.\end{aligned} \quad [3]$$

An alternative, equation [4], was reported by Haas et al. (2011) and used to predict MEm2:

$$\begin{aligned}\text{MEm2} = \text{FI} \times 18.4/0.005565 \times 0.006 \\ \times [1 + (2.38 - \text{LI}) \times 0.04],\end{aligned} \quad [4]$$

where FI represents daily feed intake of cows and LI is the level of intake or multiples of the maintenance intake level. Feed intake for first-parity cows was calculated based on formula [5], provided by Schwarz and Gruber (1999):

$$\begin{aligned}\text{FI} = 15.28 + 0.008 \times (\text{BW} - 603) + 0.2389 \\ \times (\text{ECM} - 20) - 0.005874 \times (\text{ECM} - 20)^2 + 0.305 \\ \times (\text{CON} - 2.88) + 0.959 \times (\text{ECR} - 5.41) - 0.0028 \\ \times (\text{DIM} - 112) + 1.142 \times [\ln(\text{DIM}) - 4.33] + 0.0443 \\ \times (\text{MON} - 6.36) - 0.019776 \times (\text{MON} - 6.36)^2,\end{aligned} \quad [5]$$

where **CON** represents the intake of concentrates (in kg of DM/d), **ECR** is the energy content of roughage, and **MON** is the month of lactation varying from 1 (January) to 12 (December). Because CON and ECR are difficult to measure for individuals, both variables were simulated on the basis of assumed intraherd feeding strategies. Feeding strategies are characterized by levels of energy and protein of the feeding ration. The feeding ration itself is reflected by contents for Pro% and MUN of milk samples (König et al., 2008). High Pro% combined with low values for MUN characterize a feeding strategy with high levels of concentrates, but reduced roughage supply. In contrast, low Pro% combined with high values for MUN is an indicator of concentrate limitations and increased intake of roughage. Thus, 41 herds were categorized into the 4 different feeding strategies: high or low Pro% combined with either high or low levels of MUN (Supplementary Table S1; http://dx.doi.org/10.3168/jds.2014-8618). Adaptation to low-input practices based on guidelines from Bio Suisse (2008) for organic farming in Switzerland was realized via simulation strategies. 10% of concentrates in the feeding ration (maximal tolerated level of concentrates) were assigned to cows located in farms with an average value for Pro% larger than the overall mean for Pro% (mean from all 41 participating herds). Otherwise, cows were fed without concentrates. Cows from farms with an averaged MUN level larger than the overall mean of MUN received ECR of 6.0 MJ/kg; otherwise ECR was 4.5 MJ/kg. Individual intake of concentrates for cows from the farms with the 10% concentrate feeding strategy were sampled from a normal distribution with a mean of 1.781 and standard deviation of 0.411 (Notz et al., 2013). Maximum (6.0 MJ/kg) and minimum (4.5 MJ/kg) values for ECR were fixed using real data from a sample of herds. Descriptive statistics for all test-day production traits (MY, Fat%, Pro%, and MUN), for the predicted test-day methane emissions (MEm1 and MEm2), for reproduction traits (CI, DO, and SB), and for the health trait (CM) of first-parity cows are given in Table 1.

**Table 1.** Descriptive statistics for test-day production traits, test-day methane emissions (MEm), reproduction traits, and clinical mastitis of first parity cows

| Trait | Record (n) | Cow (n) | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|---|
| Milk yield (kg) | 7,804 | 916 | 19.23 | 4.44 | 2.00 | 35.50 |
| Fat percentage (%) | 7,781 | 916 | 4.03 | 0.56 | 1.50 | 9.65 |
| Protein percentage (%) | 7,783 | 916 | 3.39 | 0.31 | 2.48 | 5.82 |
| MUN (mg/dL) | 7,781 | 916 | 25.11 | 8.25 | 5.00 | 61.00 |
| MEm1[1] (Mcal) | 7,804 | 916 | 3.35 | 0.28 | 2.17 | 4.46 |
| MEm2[1] (g) | 7,781 | 916 | 280.61 | 20.32 | 157.64 | 337.83 |
| Calving interval (d) | 713 | 713 | 387.94 | 60.57 | 273 | 664 |
| Days open | 850 | 850 | 98.22 | 60.57 | 20 | 370 |
| Stillbirth (0 or 1) | 835 | 835 | 0.05 | 0.21 | 0 | 1 |
| Clinical mastitis (0 or 1) | 911 | 911 | 0.11 | 0.32 | 0 | 1 |

[1]MEm1 = $(10.0 + 4.9 \times MY + 1.5 \times BW^{0.75}) \times 0.0132$, and MEm2 = $FI \times 18.4/0.005565 \times 0.006 \times [1 + (2.38 - LI) \times 0.04]$, where MY is milk yield, $BW^{0.75}$ is metabolic BW, FI is feed intake, and LI is level of intake.

### *Statistical Models*

Bivariate animal models were used to estimate genetic parameters during lactation for all combinations of MEm1 and MEm2 with longitudinal test-day production traits, with reproduction traits, and with CM. The AI-REML algorithm as implemented in the package DMU (Madsen and Jensen, 2012) was applied to all bivariate models.

***Model 1: Bivariate Random Regression Models for 2 Longitudinal Traits.*** For the estimation of genetic (co)variance components between MEm1 and MEm2 with test-day production traits, bivariate random regression models (**RRM**) were applied. The time-dependent covariate was DIM, altering on a continuous scale from 1 to 305 d after calving. In matrix notation, the statistical RRM for both longitudinal traits was defined as follows (longitudinal traits are indicated with index 1):

$$\mathbf{y}_1 = \mathbf{X}_1\mathbf{b}_1 + \mathbf{Z}_1\mathbf{a}_1 + \mathbf{W}_1\mathbf{p}_1 + \mathbf{e}_1,$$

where $\mathbf{y}_1$ was a vector of records for predicted test-day MEm (MEm1 or MEm2) and for a test-day production trait (MY, Fat%, Pro%, or MUN); $\mathbf{b}_1$ was a vector of fixed effects including herd, test-year-season, and third-order Legendre polynomial regressions on DIM; $\mathbf{a}_1$ and $\mathbf{p}_1$ were vectors of additive genetic and permanent environmental effects, respectively, for random regression coefficients using second-order Legendre polynomials; and $\mathbf{e}_1$ is a vector of random residual effects. $\mathbf{X}_1$, $\mathbf{Z}_1$, and $\mathbf{W}_1$ were incidence matrices for $\mathbf{b}_1$, $\mathbf{a}_1$, and $\mathbf{p}_1$, respectively. Random effects were assumed to follow a normal distribution with zero means. The variance-covariance structure for random effects was:

$$\text{var}\begin{bmatrix}\mathbf{a}\\ \mathbf{p}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{G}\otimes\mathbf{A} & 0 & 0\\ 0 & \mathbf{P}\otimes\mathbf{I}_\mathbf{p} & 0\\ 0 & 0 & \mathbf{R}\otimes\mathbf{I}_\mathbf{n}\end{bmatrix},$$

where $\mathbf{G}$ and $\mathbf{P}$ were $6 \times 6$ (co)variance matrices of random regression coefficients for the additive genetic and permanent environmental effects; $\mathbf{A}$ was an additive genetic relationship matrix; $\mathbf{I}_\mathbf{p}$ and $\mathbf{I}_\mathbf{n}$ were identity matrices for $\mathbf{p}$ cows and $\mathbf{n}$ observations, respectively; $\mathbf{R}$ was a $2 \times 2$ variance (matrix) for residual effects; and $\otimes$ denotes the Kronecker product.

***Model 2: Bivariate Random Regression and Single-Trait Models for Genetic Analyses of 1 Longitudinal Trait 1 with a Single Trait.*** For estimating genetic (co)variance components between MEm1 and MEm2 with reproduction traits (CI, DO, and SB) and with CM in consecutive bivariate runs, again RRM were applied to daily MEm1 and MEm2. Single-trait animal models were used for traits without repeated measurements (i.e., fertility and health data, and indicated with index 2). Generalized linear mixed model equations with a logit link function were applied to binary traits SB and CM. The bivariate model in matrix notation was:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix} = \begin{bmatrix}\mathbf{X}_1\mathbf{b}_1 + \mathbf{Z}_1\mathbf{a}_1 + \mathbf{W}_1\mathbf{p}_1 + \mathbf{e}_1\\ \mathbf{X}_2\mathbf{b}_2 + \mathbf{Z}_2\mathbf{a}_2 + \mathbf{Q}_2\mathbf{s}_2 + \mathbf{e}_2\end{bmatrix},$$

where $\mathbf{y}_1$ was a vector of longitudinal records for predicted test-day MEm1 or MEm2; $\mathbf{y}_2$ was a vector of records for CI or DO, or vector of liabilities for SB or CM; $\mathbf{b}_1$ was a vector of fixed effects for methane emissions, including herd, test-year-season, and third-order

Legendre polynomial regressions on DIM; $\mathbf{b}_2$ was a vector of fixed effects including herd, calving-year-season for CI, DO, SB, and CM, and additionally the sex of the calf for SB; $\mathbf{a}_1$ and $\mathbf{p}_1$ were vectors for additive genetic and permanent environmental effects, respectively, for random regression coefficients using second-order Legendre polynomials; $\mathbf{a}_2$ was a vector of additive genetic effects; $\mathbf{s}_2$ was a vector of random service sire effects for CI and SB; $\mathbf{e}_1$ and $\mathbf{e}_2$ were residual effects. $\mathbf{X}_1$, $\mathbf{X}_2$, $\mathbf{Z}_1$, $\mathbf{Z}_2$, $\mathbf{W}_1$, and $\mathbf{Q}_2$ were incidence matrices for $\mathbf{b}_1$, $\mathbf{b}_2$, $\mathbf{a}_1$, $\mathbf{a}_2$, $\mathbf{p}_1$, and $\mathbf{s}_2$, respectively. The (co)variance structure of the random effects was assumed as:

$$\text{var}\begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\\ \mathbf{p}_1\\ \mathbf{s}_2\\ \mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix}=\begin{bmatrix}\mathbf{g}_{11}\otimes\mathbf{A} & \mathbf{g}_{12}\otimes\mathbf{A} & 0 & 0 & 0 & 0\\ \mathbf{g}_{12}\otimes\mathbf{A} & \mathrm{g}_{22}\mathbf{A} & 0 & 0 & 0 & 0\\ 0 & 0 & \mathbf{pe}_{11}\otimes\mathbf{I_p} & 0 & 0 & 0\\ 0 & 0 & 0 & \mathrm{ss}_2\mathbf{I_s} & 0 & 0\\ 0 & 0 & 0 & 0 & \mathbf{r}_{11}\mathbf{I_n} & \mathbf{r}_{12}\mathbf{I_n}\\ 0 & 0 & 0 & 0 & \mathbf{r}_{12}\mathbf{I_n} & \mathbf{r}_{22}\mathbf{I_n}\end{bmatrix},$$

where $\mathbf{g}_{11}$ was a 3 × 3 (co)variance matrix of random regression coefficients for the additive genetic effects for methane emissions; $\mathrm{g}_{22}$ was the additive genetic variance of fertility traits or CM; $\mathbf{g}_{12}$ was the the additive genetic covariance vector between methane emissions and fertility traits or between methane emissions and CM; $\mathbf{pe}_{11}$ was a 3 × 3 (co)variance matrix of random regression coefficients for permanent environmental effects for predicted methane emissions; $\mathrm{ss}_2$ was the variance of random service sire effects for CI or SB; $\mathbf{I_s}$ was an identity matrix for $\mathbf{s}$ sires; $\mathbf{A}$, $\mathbf{I_p}$, $\mathbf{R}$, $\mathbf{I_n}$, and ⊗ were the same as described previously for the bivariate RRM.

Approximate standard errors of heritability estimates were calculated by a Taylor series expansion as reported by Fischer et al. (2004). Variance of heritability at time $i$ was calculated using the following equation:

$$\text{var}\left(\frac{g_{i,i}}{y_{i,i}}\right)=\text{var}(h_i^2)\approx \frac{y_{i,i}^2\,\text{var}(g_{i,i})+g_{i,i}^2\,\text{var}(y_{i,i})-2g_{i,i}y_{i,i}\,\text{cov}(g_{i,i},y_{i,i})}{y_{i,i}^4},$$

where $y_{i,i}=g_{i,i}+p_{i,i}+e$; $g_{i,i}$, $p_{i,i}$ and $y_{i,i}$ are diagonal elements of the genetic, the permanent environmental and total phenotypic (co)variance matrix, respectively; $e$ is residual; and $\text{var}(g_{i,i})$, $\text{var}(y_{i,i})$, and $\text{cov}(g_{i,i}, y_{i,i})$ are variances and covariances of genetic and phenotypic variances at time $i$.

Standard errors of genetic correlations were calculated as suggested by Lynch and Walsh (1998):

$$\text{var}(r_g)=(r_g^2)\left[\frac{\text{var}(g_1)}{4g_1^2}+\frac{\text{var}(g_2)}{4g_2^2}+\frac{\text{var}(g_{12})}{g_{12}^2}+\frac{\text{cov}(g_1,g_2)}{2g_1g_2}-\frac{\text{cov}\left(g_1,g_{12}\right)}{g_1g_{12}}-\frac{\text{cov}(g_{12},g_2)}{g_{12}g_2}\right],$$

where $r_g$ is genetic correlation between traits 1 and 2; $\text{var}(g_1)$ and $\text{var}(g_2)$ denote the variance of genetic variance for the 2 traits; $g_{12}$ is genetic covariance between traits 1 and 2; $\text{var}(g_{12})$ is the variance of genetic covariance between traits 1 and 2; $\text{cov}(g_1,g_2)$ is the covariance between the genetic variance of traits 1 and 2; $\text{cov}(g_1,g_{12})$ is the covariance between the genetic variance of trait 1 and the genetic covariance of the 2 traits; and $\text{cov}(g_{12},g_2)$ is the covariance between the genetic covariance of the 2 traits and the genetic variance of trait 2.

### *Breeding Program Scenarios*

Expected overall genetic gain and response to selection in MEm per generation were assessed using an R script (SIG.R; Pimentel and König, 2012); SIG.R is a selection index program that combines phenotypic and genomic information sources according to the theoretical framework as developed by Dekkers (2007). Traits in the breeding goal for a young sire included a production trait (MY), a fertility trait (DO), a health trait (CM), and a trait representing GHG emissions (MEm1). Equal economic weights per genetic standard deviation were defined for all 4 traits. Selection intensity was set to $i = 1$.

In 3 different scenarios, we varied index traits and information sources (i.e., daughter records vs. genomic information). Scenario I reflected a progeny testing program and index traits included 100 daughter records for MY and DO, 10 daughter records for CM, and alterations of 0 to 100 daughter records for MEm1. In scenario II, daughter records for MEm1 were replaced with a sires' genomic estimated breeding value (**GEBV**). In scenario III, daughter records for all 4 traits were completely neglected and young bull GEBV were available for all traits. Accuracies of GEBV in scenarios II and III varied between 0.1 and 1.0 and were always identical for all traits within 1 simulation. Characteristics describing the 3 different breeding scenarios are summarized in Table 2. Genetic and phenotypic parameters used for selection index calculations are

**Table 2.** Characteristics of the 3 breeding program scenarios

| Index trait[1] | Breeding scenario | | |
|---|---|---|---|
| | Scenario I, MEm1-MY-DO-CM | Scenario II, gMEm1-MY-DO-CM | Scenario III, gMEm1-gMY-gDO-gCM |
| Daughters (no.) | | | |
| MEm1 | 0–100 in increments of 10 | No | No |
| MY | 100 | 100 | No |
| DO | 100 | 100 | No |
| CM | 10 | 10 | No |
| Accuracy of GEBV | No | 0.1–1.0 for MEm1 | 0.1–1.0 for all traits |

[1]MEm1 = (10.0 + 4.9 × MY + 1.5 × $BW^{0.75}$) × 0.0132; DO = days open; MY = milk yield; CM = clinical mastitis; g = genomic breeding values; $BW^{0.75}$ = metabolic BW.

presented in Table 3 and reflect the genetic parameter estimates from the present study on a lactation basis.

## RESULTS AND DISCUSSION

### Predicted MEm

Predicted daily MEm1 and MEm2 (average from all cows) are represented in Figure 1. The MEm1 tended to decrease from 1 to 305 DIM, with maximal values of 3.66 Mcal/d at the beginning of the lactation (d 33), 3.29 Mcal/d at d 200, to minimal values with 3.05 Mcal/d at the end of lactation (d 260). Conversely, MEm2 was lowest at the beginning of lactation (about 225 g/d), increased sharply to 275 g/d around 75 DIM, and remained quite constant at this level until the end of lactation. Haas et al. (2011) also used equation [4] to predict MEm2 and showed a similar pattern of daily MEm during lactation. However, mean and range (Table 1) for predicted test-day MEm2 in the low-input Brown Swiss population differed from those predictions by Haas et al. (2011) for Holstein-Friesian cows kept in conventional production systems. The lower MEm2 of Brown Swiss cows compared with Holstein-Friesian might be attributed to the general breed effect (i.e., a lower level of milk yield; Dechow et al., 2007), lighter BW (Ozkaya and Bozkurt, 2009), and lower feed intake (Carroll et al., 2006). Milk yield, especially, has direct effect on predicted daily MEm (Garnsworthy et al., 2012b). Relationships between organic or low-input production system characteristics and production levels were carefully outlined by Nauta et al. (2006) and Yin et al. (2012). The comparably high level of MUN, with an average value of 25.11 mg/dL for cows in organic and low-input farms in Switzerland (Table 1), reflects diets with a high amount of degradable protein and deficiencies in fermentable carbohydrates (Yin et al., 2012). Such feeding rations contribute to lower MEm because retention and fermentation time is shorter compared with diets with a high fraction of carbohydrates. As outlined by Garnsworthy et al. (2012b), results of the present study confirm the strong dependency of predicted daily MEm and the equation used for the prediction. Nevertheless, phenotypic correlations between MEm1 and MEm2 at identical test days were generally larger than 0.80 and significantly different from zero (Table 4). The lowest phenotypic correlation between MEm1 and MEm2 was 0.82 for the time interval directly after calving including DIM 1 to 30 and was highest for the interval from DIM 271 to 305, with a correlation coefficient of 0.91.

**Table 3.** Estimated phenotypic variances, heritabilities (diagonal), and genetic (above diagonal) and phenotypic (below diagonal) correlations between traits on a lactation basis as used for selection index calculations

| Item | Trait | | | |
|---|---|---|---|---|
| | MEm1 | MY | DO | CM |
| Methane emissions[1] (MEm1) | 0.44 | 0.89 | 0.86 | 0.03 |
| Milk yield (MY) | 0.92 | 0.34 | 0.93 | 0.04 |
| Days open (DO) | 0.10 | 0.12 | 0.03 | −0.18 |
| Clinical mastitis (CM) | 0.02 | 0.01 | 0.02 | 0.10 |
| Phenotypic variance | 0.05 | 8.30 | 3,668.64 | 3.65 |

[1]MEm1 = (10.0 + 4.9 × MY + 1.5 × $BW^{0.75}$) × 0.0132, where $BW^{0.75}$ is metabolic BW.

### Genetic Parameters for Daily MEm

Figure 2 depicts daily heritabilities with corresponding SE for MEm1 and MEm2 from the bivariate RRM. Daily heritabilities for both traits were in a moderate range from 0.15 to 0.37 during first lactation. Daily heritability for MEm2 was 0.17 at the beginning of lactation, and increased to a maximum value of 0.30 at 128 DIM. Afterward, between 150 and 305 DIM, heritability decreased to a minimum value of 0.15. Interestingly, and despite the phenotypic differences, daily heritabilities for MEm1 and MEm2 followed an identical pattern. One obvious difference was the peak of daily

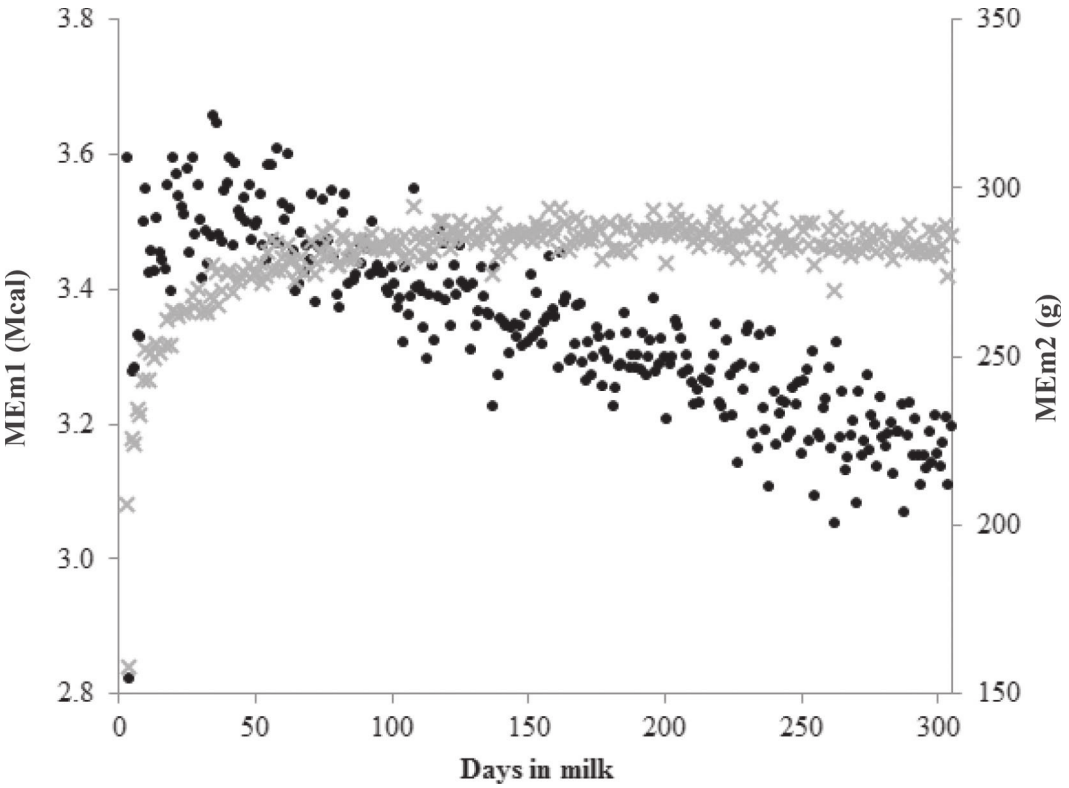


**Figure 1.** Predicted daily methane emissions (average from all cows) for MEm1 (●) and for MEm2 (×) in the course of lactation. MEm1 = (10.0 + 4.9 × MY + 1.5 × $BW^{0.75}$) × 0.0132, and MEm2 = FI × 18.4/0.005565 × 0.006 × [1 + (2.38 × LI) × 0.04], where MY is milk yield, $BW^{0.75}$ is metabolic BW, FI is feed intake, and LI is level of intake.

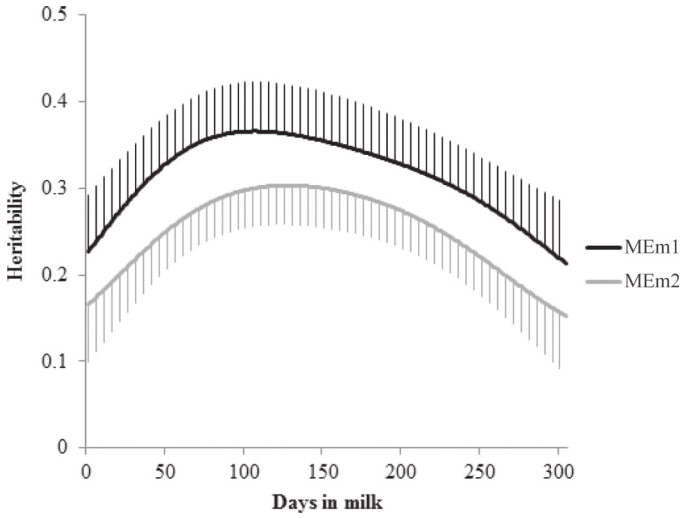


**Figure 2.** Daily heritabilities for methane emissions MEm1 (black line; SE in the range of 0.05 to 0.06) and MEm2 (gray line; SE in the range of 0.05 to 0.06). MEm1 = (10.0 + 4.9 × MY + 1.5 × $BW^{0.75}$) × 0.0132, and MEm2 = FI × 18.4/0.005565 × 0.006 × [1 + (2.38 × LI) × 0.04], where MY is milk yield, $BW^{0.75}$ is metabolic BW, FI is feed intake, and LI is level of intake.

heritabilities for MEm2, which was 30 d earlier compared with the maximal heritability for MEm1. Moderate heritabilities from the present study (i.e., average across lactation of 0.31 for MEm1 and 0.25 for MEm2) were in line with heritabilities for predicted MEm reported by Haas et al. (2011) for Holstein-Friesian and

**Table 4.** Phenotypic correlations between MEm1 and MEm2[1] within test-day intervals

| DIM | Observations (n) | Correlation | SE | *P*-value |
|---|---|---|---|---|
| 1–30 | 697 | 0.822 | 0.021 | <0.001 |
| 31–60 | 814 | 0.845 | 0.019 | <0.001 |
| 61–90 | 797 | 0.884 | 0.017 | <0.001 |
| 91–120 | 799 | 0.892 | 0.016 | <0.001 |
| 121–150 | 802 | 0.886 | 0.016 | <0.001 |
| 151–180 | 804 | 0.898 | 0.016 | <0.001 |
| 181–210 | 785 | 0.888 | 0.016 | <0.001 |
| 211–240 | 783 | 0.890 | 0.016 | <0.001 |
| 241–270 | 788 | 0.898 | 0.016 | <0.001 |
| 271–305 | 712 | 0.905 | 0.016 | <0.001 |

[1]MEm1 = (10.0 + 4.9 × MY + 1.5 × $BW^{0.75}$) × 0.0132, and MEm2 = FI × 18.4/0.005565 × 0.006 × [1 + (2.38 − LI) × 0.04], where MY is milk yield, $BW^{0.75}$ is metabolic BW, FI is feed intake, and LI is level of intake.

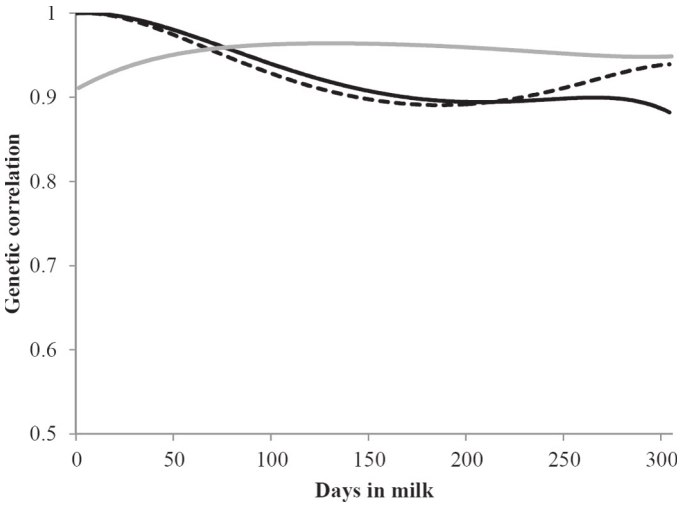


**Figure 3.** Daily genetic correlations between MEm1 and MEm2 (solid gray line, SE in the range of 0.02 to 0.09), genetic correlations between daily MEm1 with MEm1 from d 5 (solid black line; SE in the range of 0 to 0.14), genetic correlations between daily MEm2 with MEm2 from d 5 (dashed black line; SE in the range of 0 to 0.19). MEm1 = $(10.0 + 4.9 \times MY + 1.5 \times BW^{0.75}) \times 0.0132$, and MEm2 = $FI \times 18.4/0.005565 \times 0.006 \times [1 + (2.38 \times LI) \times 0.04]$, where MY is milk yield, $BW^{0.75}$ is metabolic BW, FI is feed intake, and LI is level of intake.

for real MEm recorded in ewes (Pinares-Patiño et al., 2011). Cassandro et al. (2010) based the prediction equation on DMI of Holstein-Friesian cows and found a lower heritability (0.12) for predicted MEm.

Daily genetic correlations between MEm1 and MEm2 at identical test days were higher than 0.90 throughout lactation with small SE in the range from 0.01 to 0.04 (Figure 3). The correlation increased from 0.91 to 0.96 between DIM 1 and 60 and was 0.96 for later lactation stages. High phenotypic and genetic relationships at identical test days indicate that MEm1 and MEm2 can be considered as identical traits for genetic evaluations. Figure 3 also displays genetic correlations between individual DIM and 5 DIM for both measurements MEm1 and MEm2. As expected, genetic correlations between adjacent DIM were higher than those for DIM with a greater distance. Similar results were shown in previous studies for production and functional traits (e.g., de Roos et al., 2004; Karacaören et al., 2006). For MEm1, genetic correlations between individual DIM and 5 DIM were close to 1 in the beginning of lactation, followed by a gradually decrease to 0.89 at 210 DIM, and were lowest (0.88) at the very end of lactation (DIM 305). The same trend was found for MEm2, except that genetic relationships slightly increased to 0.94 from 200 to 305 DIM. High genetic correlations in the same trait between different DIM indicate that MEm measured at different time points of lactation might be genetically the same trait. High genetic correlations imply identical ranks of sires according to EBV for MEm over lactation. Garnsworthy et al. (2012b) also found consistency when ranking dairy cows according to MEm across lactation. For predicted MEm, Haas et al. (2011) reported a substantially lower genetic correlation of 0.36 between predictions early in lactation (wk 1 to 5) with predictions late (wk 26 to 30) in lactation.

### *Genetic Correlations Between Daily MEm and Test-Day Production Traits*

Daily genetic correlations between MEm2 and test-day MY ranged between 0.70 and 0.92 (Figure 4), indicating an antagonistic genetic relationship between MEm and productivity. Standard errors of genetic correlation estimates gradually increased from 0.03 to 0.16 from the beginning to the end of lactation. The bivariate RRM model, including MEm1 and MY, did not converge for a strict convergence criterion. An explanation might be the autocorrelation between both traits. In this context, an autocorrelation means a strong effect of MY on predicted MEm1. Nevertheless, a strong association between milk productivity per cow and resource efficiency in terms of reduced MEm per kilogram of milk was emphasized in previous studies (e.g., Flachowski and Brade, 2007). Positive genetic correlations between predicted MEm with fat- and with protein-corrected milk production were also reported by Haas et al. (2011). However, estimates by Haas et al. (2011) were lower than those in the present study and ranged from 0.19 at the beginning to 0.58 at the end of lactation, presumably due to the effect of precorrection for MY. Genetic correlations between MEm1 and Fat% differed from correlations between MEm2 and Fat%. For example, slightly positive genetic correlations were found for MEm2 with Fat% from 100 to 220 DIM (genetic correlation ranged from 0.11 to 0.25), but genetic relationships were negative when correlating MEm1 with Fat% within the same time interval (genetic correlation ranged from −0.30 to −0.23). Negative genetic correlations between MEm2 and Fat% were observed at the beginning and at the end of lactation. Likewise, genetic correlations between MEm1 and Fat% were lowest (−0.57) at the end of lactation. Negative genetic relationships between MEm1 and Fat% across lactation indicate that selection on increasing Fat% is associated with desired effects on reduced MEm. In contrast, genetic relationships between MEm2 and Fat% were positive in the middle of lactation. Variation of genetic correlations during lactation indicates changes of genetic or of physiological mechanisms. For example, high Fat% early in lactation is mostly due to mobilization of body

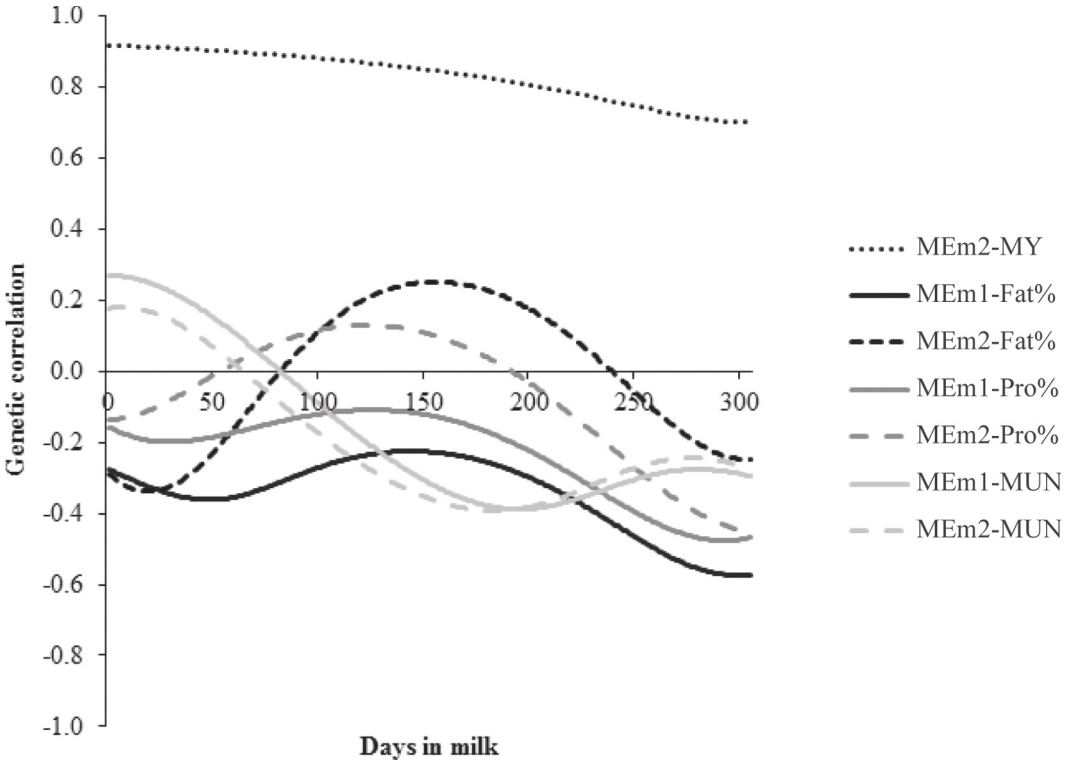


**Figure 4.** Daily genetic correlations between MEm1 and MEm2 with test-day milk yield (MY), fat percentage (Fat%), protein percentage (Pro%), and MUN. MEm1 = (10.0 + 4.9 × MY + 1.5 × $BW^{0.75}$) × 0.0132, and MEm2 = FI × 18.4/0.005565 × 0.006 × [1 + (2.38 × LI) × 0.04], where $BW^{0.75}$ is metabolic BW, FI is feed intake, and LI is level of intake. Standard errors of daily genetic correlations were in the following range: 0.04 to 0.16 (MEm2 with MY), 0.02 to 0.11 (MEm1 with Fat%), 0.01 to 0.09 (MEm2 with Fat%), 0.01 to 0.10 (MEm1 with Pro%), 0.01 to 0.12 (MEm2 with Pro%), 0.01 to 0.18 (MEm1 with MUN), and 0.01 to 0.12 (MEm2 with MUN).

fat depots (Toni et al., 2011), but strongly determined by the composition and ingredients of the feeding ration in the middle of lactation. Also, genetic variances and heritabilities for Fat% varied substantially across lactation, with the lowest heritability (0.15) at 64 DIM.

Curves for daily genetic correlations between Pro% and MEm1, and between Pro% and MEm2, showed a similar shape across lactation with maximal values in the middle of lactation. Genetic correlations between MEm and Pro% close to zero at the beginning of lactation imply that selection on MEm does not influence Pro% on the genetic scale and vice versa. Genetic correlations between MEm predictions with Pro% were favorably negative in the last third of lactation (−0.45 to −0.20) and in agreement with selection strategies aiming on improved Fat%.

Throughout lactation, genetic correlations between MEm1 and MUN were almost identical with correlations between MEm2 and MUN at corresponding test days. Genetic correlations declined from 0.20 directly after calving to −0.40 in the peak phase of lactation (200 DIM), and were negative (<−0.20) in the last lactation stage. Yin et al. (2012) found a high level of MUN in organic and low-input herds and attributed these findings to the lower percentage of concentrates in the feeding ration. Reduction of concentrates is strongly associated with a lack of dietary energy, but energy is required for the activation of ruminal microbacteria to metabolize urea into protein. With regard to the first 50 DIM, reduction of MUN is also associated with decreasing MEm on the genetic scale. Differences might be due to the interplay between breeding and feeding. From the middle to the end of lactation, direct genetic selection on increasing MUN indirectly reduces MEm. However, a breeding strategy on increasing MUN is associated with impaired female fertility (König et al., 2008) and indicates energy deficiency of lactating cows (Roy et al., 2011) with associated risks of subsequent health problems. A high concentration of ammonia due to metabolism of excess dietary protein might even be toxic to animal tissues (Rajala-Schultz et al., 2001). From an environmental perspective, an increase in

MUN is associated with aggravated ammonia emissions into the atmosphere (Powell et al., 2011).

### Genetic Correlations Between Daily MEm and Reproduction Traits

Genetic correlations between predicted MEm and both female fertility traits DO and CI showed an identical pattern across lactation (Figure 5) and illustrate the close relationship between both interval traits DO on CI (Silva et al., 1992), also on the genetic scale. High genetic correlations, especially at the end of lactation (0.93 between MEm2 and DO and 0.85 between MEm2 and CI at 305 DIM), indicate resource inefficiency for cows with impaired female fertility. Positive genetic correlations between predicted MEm and fertility traits (DO and CI) imply that breeding on shorter CI, shorter DO, and lower MEm can be achieved simultaneously. Hence, without access to phenotypic data for MEm (e.g., measurements from a respiration chamber or from a mobile laser methane detector), and without availability of proper indicator traits for MEm predictions (e.g., BW), we suggest a breeding strategy emphasizing female fertility traits. As a side effect, improved cow fertility reduces within-herd replacement rates. As a consequence, reduced replacements also contribute to decreasing methane emissions (Knapp et al., 2014).

Apart from the end of lactation, methane emissions (MEm2) were favorably genetically correlated with the functional trait SB. To our knowledge, no studies have addressed relationships between GHG emissions and SB. In general, results from the present study support a breeding strategy on functional female fertility traits as considered in current breeding goals for dairy cattle. The bivariate linear-threshold model, including longitudinal test-day MEm1 and categorical SB, did not converge. Generally, standard errors of genetic correlations between MEm2 and SB were quite large (0.189 at the end of lactation). This might be due to the binary outcome of SB combined with a low number of records. For Gaussian as well as for categorical traits,

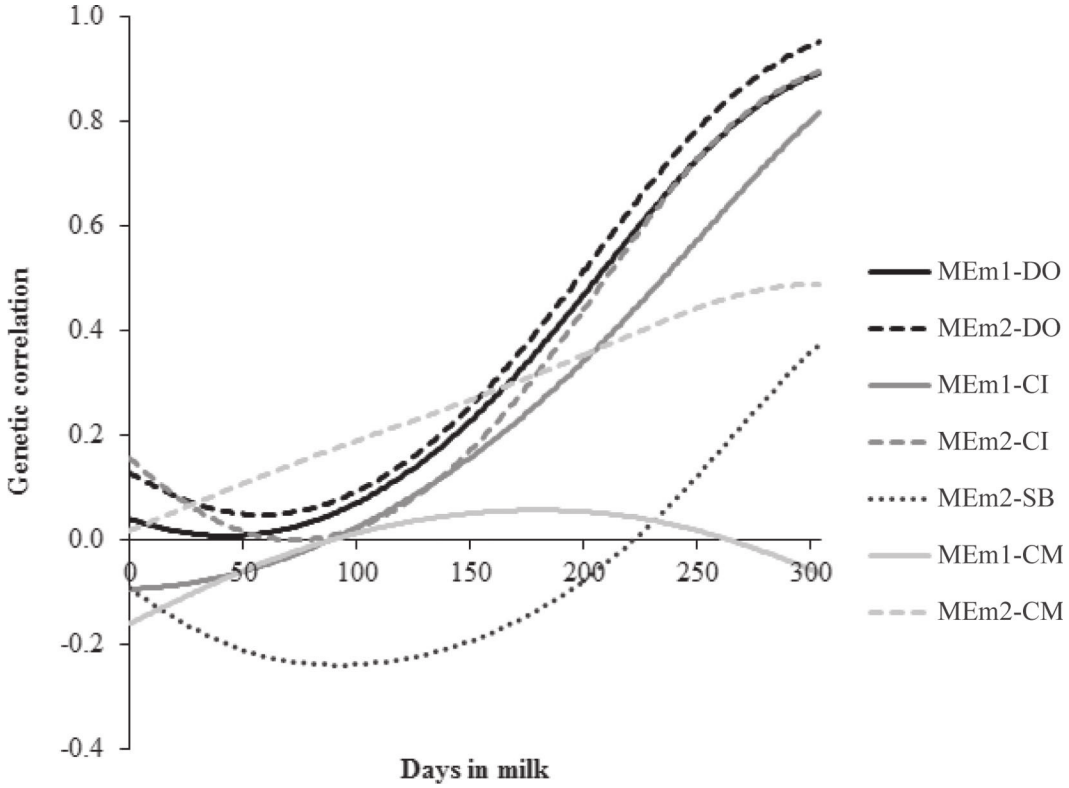


**Figure 5.** Daily genetic correlation between MEm1 and MEm2 with days open (DO), calving interval (CI), stillbirth (SB), and clinic mastitis (CM). MEm1 = $(10.0 + 4.9 \times MY + 1.5 \times BW^{0.75}) \times 0.0132$, and MEm2 = $FI \times 18.4/0.005565 \times 0.006 \times [1 + (2.38 \times LI) \times 0.04]$, where MY is milk yield, $BW^{0.75}$ is metabolic BW, FI is feed intake, and LI is level of intake. Standard errors of daily genetic correlations were in the following range: 0.001 to 0.11 (MEm1 with DO), 0.003 to 0.14 (MEm2 with DO), 0.001 to 0.11 (MEm1 with CI), 0.001 to 0.16 (MEm2 with CI), 0.002 to 0.18 (MEm2 with SB), 0.001 to 0.07 (MEm1 with CM), and 0.002 to 0.19 at DIM 305 (MEm2 with CM).

standard errors were largest at the extreme ends of the time scale.

### Genetic Correlations Between MEm and CM

Direct breeding strategies on dairy cattle udder health contribute to reduce MEm. In our study, genetic correlations between MEm2 and CM were positive, and thus favorable, in the course of lactation, with a maximal value of 0.38 at 305 DIM (Figure 5). Rehbein et al. (2013) focused on the relationships between female fertility traits and CM on the phenotypic and on the genetic scale by applying structural equation models. They found longer interval traits and lower success of a first insemination for cows with udder infections, and they discussed the underlying physiological mechanisms. Hence, breeding on reduced MEm not only will improve a cow's fertility status, but also contribute to udder health. Due to moderate heritabilities for MEm and the favorable correlations with functional traits, it might be worthwhile to implement recording technique to measure MEm of dairy cows on a farm-gate level. Conversely, the implementation of a recording technique for health traits not only generates a basis for health trait genetic evaluations, but also for genetic selection with a focus on MEm.

### Limitations of Genetic Statistical Analysis Based on Simulated Data

The present approach to predict daily MEm strongly relies on the parameters and traits used in both prediction equations. Furthermore, some indicator traits for predicting MEm were calculated based on a second function. Specifically, FI was used to predict MEm, but without direct measurements for FI; thus, FI itself was calculated including ECM, CON, DIM, MON, and ECR. Stochastic simulations were applied to simulate ECR, but only taking into account variants of MUN and Pro% as indicators for general feeding strategies and a random error component. In practice, a broader variety of effects contribute to phenotypic variations of ECR. The ECR itself is a major component used as a basis for prediction equations, and, as a consequence, we assume reduced variations for FI and MEm when comparing to real data. Also, MEm1 is a prediction function only describing that part of MEm which is dependent on the factors and effects included in the function. Likely, more important parts contribute to variations of MEm that are not explained or are independent from these functions. The smallest values for coefficients of variations for MEm1 and MEm2 when comparing to other traits underline this theoretical concept based on functions and parameters of the functions. In a strict sense, genetic parameters do not reflect the genetic background of MEm but rather for the functions of other traits that were used to predict MEm. Nevertheless, MEm is a novel trait of increasing importance, but cannot be recorded on population-wide scales. This approach is a further alternative to get first insight into genetic components of MEm using traits and parameters from official recording schemes, and supporting previous quantitative genetic studies based on predicted MEm (e.g., Haas et al., 2011).

### Breeding Strategies

Overall genetic gain and response to selection in MEm1 per generation are shown in Figures 6 and 7, respectively. When increasing the number of daughters for MEm1 in a pure progeny testing program (scenario I), only a marginal effect on overall genetic gain in complex breeding goals including several traits was observed. This is particularly the case for large daughter groups for other traits (i.e., assumed 100 daughter records per sire for both conventional traits MY and DO). Also, antagonistic genetic relationships among traits used in indices and breeding goals hamper selection response in individual traits. In the present study, pronounced antagonistic relationships were found and modeled between MY and DO (genetic correlation = 0.93). König et al. (2013) confirmed those findings via the application of selection index methodology to conventional indices (indices without genomic information) and to genomic indices stepwise by including additional traits into the overall breeding goal.

Both evaluation criteria, overall genetic gain and selection response for MEm1 per generation, clearly exhibited a strong increase with increasing accuracies of GEBV for the pure genomic breeding strategy (scenario III). Genetic gain from scenario III is higher than genetic gain from the progeny testing scenario I for accuracies of GEBV larger than 0.80. Such a crucial threshold for GEBV was identified in previous studies (e.g., Pimentel and König, 2012) and is achieved in dairy cattle breeding programs when basing genomic selection on large calibration groups of sires. However, for novel traits such as MEm, it is imperative to set up a calibration group of cows (Buch et al., 2012; Pszczola et al., 2012). Deterministic equations (e.g., Goddard, 2009; Daetwyler et al., 2010) were developed to determine the required calibration group size for desired accuracies of GEBV. For the moderate heritability trait MEm1 ($h^2 = 0.44$), assuming an effective population size $N_e = 100$, and applying the deterministic prediction by Daetwyler et al. (2010), a desired accuracy of

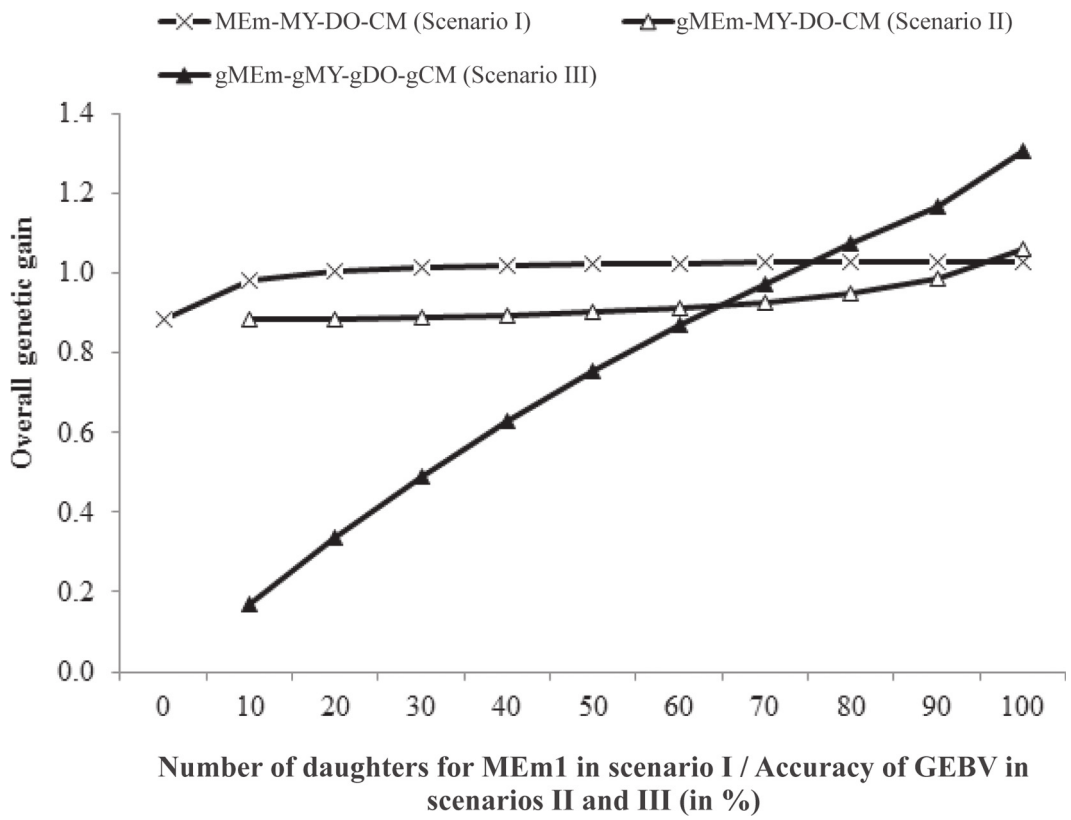


**Figure 6.** Genetic gain per generation for scenarios I, II, and III (as explained in Table 2). MEm1 = $(10.0 + 4.9 \times MY + 1.5 \times BW^{0.75}) \times 0.0132$; MY = milk yield; DO = days open; CM = clinical mastitis; g = genomic breeding values; $BW^{0.75}$ = metabolic BW.

0.80 for GEBV implies a calibration group including 2,581 cows. Logistically, phenotyping and genotyping of 2,581 cows might be easier to realize compared with the implementation of a progeny testing program for a novel trait MEm. In Germany, more than 20,000 cows from large-scale contract herds located in the eastern part of Germany are the basis for genomic selection with a focus on low heritability health traits (Martin et al., 2013). A fraction of those cows can be used for direct or indirect MEm measurements. However, a cow calibration group in the Brown Swiss low-input population only included 1,126 cows (Kramer et al., 2014); therefore, accuracies of GEBV for low-heritability functional traits were low to moderate. Following Daetwyler et al. (2010), the expected accuracy of GEBV is 0.66 for a calibration group size of 1,126 cows ($h^2$ = 0.44, and $N_e$ = 100). König and Swalve (2009) applied selection index methodology for the calculation of the required number of daughters per genotyped sire to achieve predefined correlations between the index and the aggregated genotype ($r_{TI}$) by altering heritabilities and accuracies of GEBV ($r_{mg}$). For a desired aggregated genotype of 0.95, for heritability = 0.45, and for accuracies of GEBV = 0.70, at least 66 additional daughters have to be included in genetic evaluations. From a logistic perspective, Schierenbeck et al. (2011) suggested the implementation of a contract herd system to ensure a combination of novel traits with cow genotypes. Furthermore, a contract herd system allows specific strategies for the use of sires and, as a consequence, the setting up of specific genetic structures being suitable for imputation procedures (Pimentel et al., 2013).

## CONCLUSIONS

Longitudinal daily MEm can be predicted when combining real data with deterministic equations and stochastic simulations. Moderate heritabilities were found for predicted MEm over lactation. Due to the strong genetic correlations with production traits, we suggest indirect selection strategies on routinely recorded test-day MY, Fat%, and Pro% by simultaneously reducing MEm. Nevertheless, autocorrelations exist because MY was used as a fundamental component in predicting MEm. The positive genetic correlations between fertility traits (DO and CI) and MEm indicates that

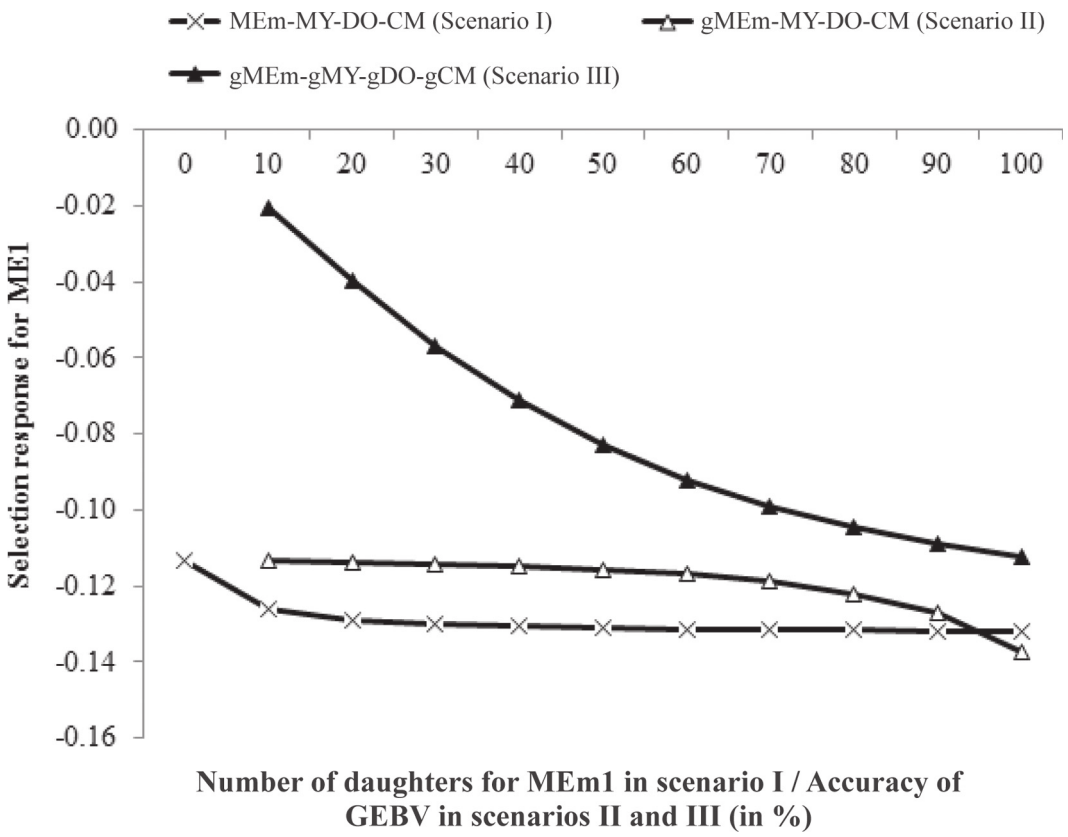


**Figure 7.** Response to selection per generation for methane emissions (MEm1 = (10.0 + 4.9 × MY + 1.5 × $BW^{0.75}$) × 0.0132, where MY is milk yield, $BW^{0.75}$ is metabolic BW) for scenarios I, II, and III (as explained in Table 2). MY = milk yield; DO = days open; CM = clinical mastitis; g = genomic breeding values.

selection for better fertility of cows likewise reduces MEm. Genetic correlations between MEm and CM were positive, especially at the end of lactation, and underline the importance of health data recording at a large scale. Genetic (co)variance components for MEm might be biased because the prediction functions only describe that part of MEm which is dependent on the factors and effects included in the function. Therefore, it is necessary to evaluate the reliability of the predict equation based on real MEm measurements.

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## 2.4. Original research paper 4

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# Genetic parameters for body weight from birth to calving and associations between weights with test-day, health, and female fertility traits

**Tong Yin and Sven König**[1]
Institute of Animal Breeding and Genetics, Justus-Liebig-University Gießen, 35390 Gießen, Germany

## ABSTRACT

A data set including 57,868 records for calf birth weight (CABW) and 9,462 records for weight at first insemination (IBW) were used for the estimation of direct and maternal genetic effects in Holstein Friesian dairy cattle. Furthermore, CABW and IBW were correlated with test-day production records and health traits in first-lactation cows, and with nonreturn rates in heifers. Health traits considered overall disease categories from the International Committee for Animal Recording diagnosis key, including the general disease status, diarrhea, respiratory diseases, mastitis, claw disorders, female fertility disorders, and metabolic disorders. For single-trait measurements of CABW and IBW, animal models with maternal genetic effects were applied. The direct heritability was 0.47 for CABW and 0.20 for IBW, and the direct genetic correlation between CABW and IBW was 0.31. A moderate maternal heritability (0.19) was identified for CABW, but the maternal genetic effect was close to zero for IBW. The correlation between direct and maternal genetic effects was antagonistic for CABW (−0.39) and for IBW (−0.24). In bivariate animal models, only weak genetic and phenotypic correlations were identified between CABW and IBW with either test-day production or health traits in early lactation. Apart from metabolic disorders, there was a general tendency for increasing disease susceptibilities with increasing CABW. The genetic correlation between IBW and nonreturn rates in heifers after 56 d and after 90 d was slightly positive (0.18), but close to zero when correlating nonreturn rates with CABW. For the longitudinal BW structure from birth to the age of 24 mo, random regression models with the time-dependent covariate "age in months" were applied. Evaluation criteria (Bayesian information criterion and residual variances) suggested Legendre polynomials of order 3 to modeling the longitudinal body weight (BW) structure. Direct heritabilities around birth and insemination dates were slightly larger than estimates for CABW and IBW from the single-trait models, but maternal heritabilities were exactly the same from both models. Genetic correlations between BW were close to 1 for neighboring age classes, but decreased with increasing time spans. The genetic correlation between BW at d 0 (birth date) and at 24 mo was even negative (−0.20). Random regression model estimates confirmed the antagonistic relationship between direct and maternal genetic effects, especially during calfhood. This study based on a large data set in dairy cattle confirmed genetic parameters and (co)variance components for BW as identified in beef cattle populations. However, BW records from an early stage of life were inappropriate early predictors for dairy cow health and productivity.

**Key words:** body weight, health and fertility trait, genetic parameter

## INTRODUCTION

Body weight of dairy cattle is a novel trait of increasing economic importance, because BW change indicates maintenance requirements of lactating cows and growing heifers, determines the carcass values of cows, and is associated with weight development in offspring (Byrne et al., 2016). Energy balance modeling via BW changes is also important from a breeding perspective, especially during the early lactation stage directly after calving (Coffey et al., 2002). The negative energy balance impairs health and fertility (Collard et al., 2000), as well as productivity in the ongoing and later lactations of milking cows (Berry et al., 2003a). Compared with other components determining energy balance (e.g., DMI, methane emissions, or $NE_M$), BW is quite easy to measure under practical on-farm conditions.

Direct heritabilities for BW from different age points reported in literature were in a moderate to high range (Table 1), indicating the potential for genetic improvements. Also moderate to high genetic correlations between BW of milking cows with DMI and energy balance (Veerkamp et al., 2000) suggest BW recording and utilization for correlated selection response. However, for production and reproduction traits, genetic

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[1]Corresponding author: sven.koenig@agrar.uni-giessen.de

correlations with BW were low and varied across studies (Table 1). For calf birth weight (**CABW**), direct heritabilities were significantly larger compared with maternal heritabilities (Everett and Magee, 1965; Johanson et al., 2011). Moderate to high positive genetic correlations between CABW with dystocia, perinatal mortality, and gestation length were reported by Johanson et al. (2011). Availability of producer diagnosis keys for cow health traits according to International Committee for Animal Recording (**ICAR**) guidelines (Stock et al., 2013), and reflecting the disease categories of claw disorders, mastitis, metabolic disorders, and female fertility disorders, allow further association studies with BW measurements. However, to our knowledge, detailed genetic analyses in this regard are lacking.

Body weight recording allows a longitudinal data structure measured at different time points, including birth weight, weaning weight, cow calving weight, or BW from different lactation stages (Lamb and Barker, 1975; Coffey et al., 2006). Generally, genetic correlations for weight measurements from time points in close distance were quite large [e.g., 0.79 between birth weight and weaning weight (Coffey et al., 2006)], and between BW in wk 1 and 15 of lactation (Veerkamp and Thompson, 1999). In contrast, genetic correlations between distant time measurements were quite small [e.g., 0.14 between BW from d 50 to 900; Brotherstone et al. (2007)]. For genetic analyses of longitudinal weight data, repeatability models (e.g., Abdallah and McDaniel, 2000), multiple trait models (e.g., Veerkamp and Thompson, 1999), or the random regression model (**RRM**, e.g., Coffey et al., 2006) can be applied. Repeatability model applications assume identical genetic and environmental variances across the given time period. An alternative is to consider repeated weight measurements from different periods as separate traits, being the data basis for multiple trait model applications (Veerkamp and Thompson, 1999). Multiple trait models allow consideration of altering additive-genetic and residual variances, with positive effects on the accuracy of genetic evaluations (Thompson and Meyer, 1986). However, in the case of a large number of traits, the multiple-trait model might be over-parameterized (Veerkamp and Thompson, 1999). In RRM, alterations of genetic parameters and breeding values over the recording trajectory can be estimated based on a limited number of random regression coefficients.

**Table 1**. Overview of heritabilities for BW traits and their genetic correlations with production, fertility, and health traits in Holstein cows

| | Heritability | | Genetic correlation | | |
|---|---|---|---|---|---|
| Trait | Direct | Maternal | Trait | Value | Reference |
| Birth weight | 0.22 | 0.04 | Gestation length | 0.57 | Everett and Magee, 1965 |
| Birth to d 36 weight | 0.58 | | | | Brotherstone et al., 2007 |
| Birth weight | 0.26 | 0.08 | Dystocia | 0.73 | Johanson et al., 2011 |
| | | | Perinatal mortality | 0.57 | |
| | | | Gestation length | 0.52 | |
| BW[1] | 0.60 | | Milk yield | −0.03 | Berry et al., 2003a |
| | | | Protein yield | 0.03 | |
| | | | Fat yield | −0.01 | |
| | | | Interval to first service | −0.25 | |
| | | | Pregnant to first service | −0.22 | |
| | | | First service to conception interval | 0.37 | |
| | | | Number of services | 0.15 | |
| BW[2] | 0.17 | | 3.7% FCM | −0.15 | Abdallah and McDaniel, 2000 |
| | | | 2×, 305-d, mature equivalent fat yield | −0.11 | |
| | | | Days open | −0.11 | |
| Birth weight | 0.53 | | Weaning weight | 0.79 | Coffey et al., 2006 |
| | | | Calving weight | 0.50 | |
| Weaning weight | 0.45 | | Calving weight | 0.59 | |
| Calving weight | 0.75 | | | | |
| Live weight[3] | 0.35 | | Fat- and protein-corrected milk | −0.10 | Lassen and Løvendahl, 2016 |
| Live weight[4] | 0.48 | | Milk yield | −0.06 | Veerkamp et al., 2000 |
| | | | Fat yield | 0.31 | |
| | | | Protein yield | 0.20 | |
| | | | DMI | 0.76 | |
| | | | Energy balance | 0.45 | |
| | | | Interval until first luteal activity | −0.11 | |

[1]Average BW from DIM 5, 60, 120, 180.

[2]Predicted BW after calving.

[3]Weekly average live weight measured by automatic milking systems.

[4]Live weight within the first week of first-lactation cows.

Apart from CABW and weaning weight, there is a gap addressing genetic investigations for weight records along the trajectory between birth and first calving. Furthermore, genetic associations between BW during aging with health and productivity are unclear. Hence, the objectives of the present study were to (1) estimate genetic parameters for CABW and body weight at first insemination (**IBW**) applying multiple trait models, (2) to infer genetic and phenotypic relationships between CABW and IBW with nonreturn rates in heifers, and with production and health traits during the early lactation period of first-parity cows, and (3) to analyze longitudinal BW records from birth to first calving applying RRM.

## MATERIALS AND METHODS

### Data

***CABW and IBW.*** The CABW was available from 57,868 animals kept in 53 large-scale contract herds located in 2 federal states of northeast Germany. The calves were born from 2005 to 2014, and CABW varied between 20 to 60 kg. No further outliers for CABW were detected. A subset of 9,462 heifers additionally had records for IBW. Heifers with IBW were extracted according to their weight date and first insemination date. If the difference between the 2 dates was shorter than 10 d, the heifer weight was considered as IBW. Insemination age of the 9,462 heifers varied from 329 to 777 d, with an average value of 452.68 d. On average, the difference between weight date and first insemination date was 5.05 d.

***Cow Production and Health Traits.*** Production and health traits were available from first-lactation cows. Analogous to Mahmoud et al. (2017), the production traits included records from the first and second official test-day after calving for milk yield (**MY1** and **MY2**), fat percentage (**FP1** and **FP2**), protein percentage (**PP1** and **PP2**), fat yield (**FY1** and **FY2**), protein yield (**PY1** and **PY2**), fat to protein ratio (**FPR1** and **FPR2**), and SCS (**SCS1** and **SCS2**).

Health traits of first-lactation cows were recorded according to the hierarchical diagnosis key as considered in the official ICAR guidelines (Stock et al., 2013). For the present analyses, the same overall disease categories as introduced by Mahmoud et al. (2017) were used. These were the general disease status (**GDS**), diarrhea (**DIA**), respiratory diseases (**RD**), mastitis (**MAST**), claw disorders (**CL**), female fertility disorders (**FF**), and metabolic disorders (**MET**). The overall disease trait category GDS reflects the first level of the hierarchical diagnosis key (i.e., just a classification if a cow is healthy or sick, without specifying the disease). The remaining disease categories used in this study (RD, MAST, CL, FF, and MET) represent the second level of the diagnosis key. The diagnosis key also has options for a detailed disease specification including, for example, the location of the disease in the case of claw disorders. However, the detailed specification system for single diseases was not used in all herds, and for some specific diseases, incidences were quite low. Hence, we decided to use the diagnosis key levels 1 and 2 with the corresponding disease categories in the present study. If one entry of the particular disease category was observed in the first lactation, a value of 1 was assigned for a sick cow. Otherwise, a 0 was assigned for a healthy cow.

***Heifer Fertility Traits.*** Female fertility traits of heifers were the nonreturn rate after 56 d (**NRR56**) and the nonreturn rate after 90 d (**NRR90**). The NRR56 and NRR90 were also defined as binary traits, with a 0 for nonpregnant heifers and a 1 for pregnant heifers. Descriptive statistics for CABW, IBW, cow production, and cow health traits, as well as reproduction traits from heifers are summarized in Tables 2 and 3.

***Longitudinal Weight Data.*** In the selected large-scale contract herds, weight recording of calves and heifers from different age stages was performed in close intervals because of automatically installed weighting technique. The CABW was available from all calves from the herd, and a large subset was recorded for IBW. A large fraction of animals was weighed shortly before first calving at an age of 24 mo. Hence, the time span for repeated measurement analyses was from birth to an age of 24 mo. Only animals with a record for CABW and 5 repeated measurements were considered. The effects of the herd, weighing year, and weighing month were combined to create a contemporary group "herd-year-month." Editing criterion was that each level of the herd-year-month effect included at least 10 weight records. Weight outliers were detected by studentized residuals, reflecting the influence of each weight record on the overall estimates. For each weight record, studentized residuals and corresponding Bonferroni $P$-values were calculated using the outlier test function in the R-package "car" (Fox and Weisberg, 2011). Records with $P$-value smaller than 0.05 or larger than 0.95 were excluded as outliers. Finally, 32,404 longitudinal weight observations from 4,952 animals were considered.

### Statistical Models

***Single Weight Measurement Analysis: Genetic Parameters for CABW and IBW.*** An animal model with maternal genetic effects was applied to estimate genetic (co)variance components for CABW and IBW.

**Table 2**. Descriptive statistics for calf birth weight, weight at first insemination, weight from 0 to 24 mo, and test-day production traits

| Trait | No. of animals[1] | Mean[2] | SD[2] | Min.[2] | Max.[2] | No. of animals[3] |
|---|---|---|---|---|---|---|
| Calf birth weight (kg) | 57,868 | 41.48 | 4.82 | 20 | 60 | |
| Weight at first insemination (kg) | 9,462 | 406.46 | 32.90 | 270 | 570 | |
| Milk yield at first test-day (kg) | 46,055 | 28.32 | 6.59 | 3.0 | 58.4 | 7,549 |
| Fat percentage at first test-day (%) | 46,055 | 4.21 | 0.78 | 1.61 | 8.49 | 7,549 |
| Fat yield at first test-day (kg) | 46,055 | 1.18 | 0.30 | 0.07 | 3.62 | 7,549 |
| Protein percentage at first test-day (%) | 46,055 | 3.26 | 0.33 | 2.21 | 4.50 | 7,549 |
| Protein yield at first test-day (kg) | 46,055 | 0.91 | 0.19 | 0.09 | 2.02 | 7,549 |
| Fat to protein ratio at first test-day | 46,055 | 1.30 | 0.24 | 0.37 | 3.26 | 7,549 |
| SCS at first test-day | 45,949 | 2.88 | 1.70 | −1.64 | 9.64 | 7,527 |
| Milk yield at second test-day (kg) | 39,652 | 32.45 | 5.98 | 3.0 | 59.4 | 6,419 |
| Fat percentage at second test-day (%) | 39,652 | 3.70 | 0.55 | 1.60 | 8.42 | 6,419 |
| Fat yield at second test-day (kg) | 39,652 | 1.19 | 0.24 | 0.07 | 2.60 | 6,419 |
| Protein percentage at second test-day (%) | 39,652 | 3.07 | 0.24 | 2.20 | 4.39 | 6,419 |
| Protein yield at second test-day (kg) | 39,652 | 0.99 | 0.17 | 0.10 | 1.80 | 6,419 |
| Fat to protein ratio at second test-day | 39,652 | 1.21 | 0.19 | 0.48 | 2.88 | 6,419 |
| SCS at second test-day | 39,620 | 2.21 | 1.65 | −2.64 | 9.64 | 6,412 |
| Weights from 0 to 24 mo (kg) | 4,952 | 253.61 | 168.66 | 20 | 742 | |

[1]No. of animals for estimating genetic correlations between calf birth weight and test-day, health, and fertility traits.

[2]Mean, SD, minimum (Min.), and maximum (Max.) values were calculated based on the number of animals in column 2.

[3]No. of animals for estimating genetic correlations between weight at first insemination and test-day, health, and fertility traits.

In matrix notation, the statistical model [1] for CABW and IBW was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zd} + \mathbf{Wm} + \mathbf{Sp_m} + \mathbf{e}, \quad [1]$$

where $\mathbf{y}$ was a vector of observations for CABW or IBW; $\mathbf{b}$ was a vector of fixed effects including herd, birth year, birth month, and gestation length for CABW, and herd, insemination year, insemination month, and age at first insemination for IBW; $\mathbf{d}$ was a vector of direct additive-genetic effects; $\mathbf{m}$ was a vector of random maternal genetic effects; $\mathbf{p_m}$ was a vector of maternal permanent environmental effects; $\mathbf{e}$ was a vector of random residual effects; and $\mathbf{X}$, $\mathbf{Z}$, $\mathbf{W}$, and $\mathbf{S}$ were incidence matrices for $\mathbf{b}$, $\mathbf{d}$, $\mathbf{m}$, and $\mathbf{p_m}$, respectively.

The equations to estimate direct heritabilities $\left(h_d^2\right)$, maternal heritabilities $\left(h_m^2\right)$, and total heritabilities $\left(h_t^2\right)$ were

$$h_d^2 = \frac{\sigma_d^2}{\sigma_d^2 + \sigma_m^2 + \sigma_{dm} + \sigma_{p_m}^2 + \sigma_e^2},$$

$$h_m^2 = \frac{\sigma_m^2}{\sigma_d^2 + \sigma_m^2 + \sigma_{dm} + \sigma_{p_m}^2 + \sigma_e^2}, \text{ and}$$

$$h_t^2 = \frac{\sigma_d^2 + 1.5\sigma_{dm} + 0.5\sigma_m^2}{\sigma_d^2 + \sigma_m^2 + \sigma_{dm} + \sigma_{p_m}^2 + \sigma_e^2},$$

where $\sigma_d^2$ was the direct genetic variance; $\sigma_m^2$ was the maternal genetic variance; $\sigma_{dm}$ was the covariance be-

**Table 3**. Descriptive statistics for incidences of health traits and for success rates (SR) of female fertility traits

| | Data set 1[1] | | Data set 2[2] | |
|---|---|---|---|---|
| Trait | No. of animals | SR/incidence (%) | No. of animals | SR/incidence (%) |
| Nonreturn rate after 56 d | 51,947 | 70.06 | 9,445 | 67.46 |
| Nonreturn rate after 90 d | 51,947 | 65.49 | 9,445 | 63.43 |
| General disease status | 57,225 | 79.24 | 9,349 | 78.77 |
| Diarrhea | 57,225 | 5.74 | 9,349 | 5.77 |
| Respiratory disease | 57,225 | 2.37 | 9,349 | 4.41 |
| Mastitis | 57,225 | 22.12 | 9,349 | 23.32 |
| Claw disorders | 57,225 | 27.25 | 9,349 | 25.20 |
| Female fertility disorders | 57,225 | 41.41 | 9,349 | 41.53 |
| Metabolic disorders | 57,225 | 1.23 | 9,349 | 1.51 |

[1]No. of animals for estimating genetic correlations between calf birth weight and health and fertility traits.

[2]No. of animals for estimating genetic correlations between weight at first insemination and health and fertility traits.

tween direct and maternal genetic effects; $\sigma^2_{p_m}$ was the variance for maternal permanent environmental effect; and $\sigma^2_e$ was the residual variance.

The equation to estimate the genetic correlation ($r_{dm}$) between direct additive genetic and maternal genetic effects was

$$r_{dm} = \frac{\sigma_{dm}}{\sqrt{\sigma^2_d \times \sigma^2_m}}.$$

***Single Weight Measurement Analysis: Genetic Parameters for Production and Health Traits.*** A linear animal model (for Gaussian test-day traits), and a threshold model (for binary health traits) was defined for first-lactation test-day production and health traits. The statistical model [2] for both trait categories was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zd} + \mathbf{e}, \qquad [2]$$

where $\mathbf{y}$ was a vector of observations for test-day production traits (MY1, FP1, FY1, PP1, PY1, MY2, FP2, FY2, PP2, PY2, FPR1, FPR2, SCS1, and SCS2), and of disease liabilities for binary health traits (GDS, DIA, RD, MAST, CL, FF, and MET); $\mathbf{b}$ was a vector of fixed effects including the herd, calving year and calving season; $\mathbf{d}$ was a vector of additive genetic effects; $\mathbf{e}$ was a vector of random residual effects; and $\mathbf{X}$ and $\mathbf{Z}$ were the incidence matrices for $\mathbf{b}$ and $\mathbf{d}$, respectively.

***Single Weight Measurement Analysis: Genetic Parameters for Nonreturn Rates.*** A threshold model [3] including the random service sire effects was defined for NRR56 and NRR90. Model [3] was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zd} + \mathbf{Ws} + \mathbf{e}, \qquad [3]$$

where $\mathbf{y}$ was a vector of insemination success rates for NRR56 and NRR90; $\mathbf{b}$ was a vector of fixed effects including the herd, insemination year, insemination month, and age at first insemination; $\mathbf{s}$ was a vector of random service sire effects; $\mathbf{X}$ and $\mathbf{W}$ were incidence matrices for $\mathbf{b}$ and $\mathbf{s}$, respectively; and $\mathbf{d}$, $\mathbf{e}$, and $\mathbf{Z}$ were the same as defined in model [2].

***Single Weight Measurement Analysis: Bivariate Models.*** A first bivariate model considered CABW and IBW simultaneously. The following covariance structure of random effects was assumed:

$$\mathbf{Var}\begin{bmatrix}\mathbf{u}\\ \mathbf{m}\\ \mathbf{p_m}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{G_d}\otimes\mathbf{A} & \mathbf{G_{dm}}\otimes\mathbf{A} & 0 & 0\\ \mathbf{G'_{dm}}\otimes\mathbf{A} & \mathbf{G_m}\otimes\mathbf{A} & 0 & 0\\ 0 & 0 & \mathbf{P_m}\otimes\mathbf{I_m} & 0\\ 0 & 0 & 0 & \mathbf{R}\otimes\mathbf{I_n}\end{bmatrix},$$

where $\mathbf{G_d}$ was a 2 × 2 (co)variance matrix for the direct additive genetic effects; $\mathbf{G_m}$ was a 2 × 2 (co)variance matrix for the maternal genetic effects; $\mathbf{G_{dm}}$ was a 2 × 2 covariance matrix between direct additive genetic and maternal genetic effects; $\mathbf{A}$ was the additive genetic relationship matrix; $\mathbf{P_m}$ was a 2 × 2 (co)variance matrix for maternal permanent environmental effects; $\mathbf{I_m}$ was an identity matrix for $m$ dams; $\mathbf{R}$ was a 2 × 2 (co)variance matrix for residual effects; $\mathbf{I_n}$ was an identity matrix for $n$ observations, and ⊗ was the direct matrix product.

In bivariate runs combining model [1] with models [2] and [3], genetic correlations were estimated between CABW or IBW with cow production and health traits, and with nonreturn rates in heifers. The following (co) variance structure of random effects was assumed:

$$\mathbf{Var}\begin{bmatrix}\mathbf{u}\\ \mathbf{m}\\ \mathbf{p_m}\\ \mathbf{s}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{G_d}\otimes\mathbf{A} & \mathbf{g_{dm}}\otimes\mathbf{A} & 0 & 0 & 0\\ \mathbf{g'_{dm}}\otimes\mathbf{A} & \boldsymbol{\sigma}^2_{\mathbf{m}}\mathbf{A} & 0 & 0 & 0\\ 0 & 0 & \boldsymbol{\sigma}^2_{\mathbf{p_m}}\mathbf{I_m} & 0 & 0\\ 0 & 0 & 0 & \boldsymbol{\sigma}^2_{\mathbf{s}}\mathbf{I_s} & 0\\ 0 & 0 & 0 & 0 & \mathbf{R}\otimes\mathbf{I_n}\end{bmatrix},$$

where $\mathbf{G_d}$ was a 2 × 2 (co)variance matrix for the direct additive genetic effects including one production, health, or fertility trait, and CABW or IBW; $\boldsymbol{\sigma}^2_{\mathbf{m}}$ was the maternal genetic variance for CABW or IBW; $\mathbf{g_{dm}}$ was a 2 × 1 vector for the covariances between direct additive genetic effects for one weight trait (CABW or IBW) and for one of the production, fertility, or disease traits with maternal genetic effects for CABW or IBW; $\boldsymbol{\sigma}^2_{\mathbf{p_m}}$ was the maternal permanent environmental variance for CABW or IBW; $\boldsymbol{\sigma}^2_{\mathbf{s}}$ was the variance for service sires; and $\mathbf{I_s}$ was an identity matrix for $s$ service sires (only relevant for NRR56 and NRR90).

For all bivariate models, the BLUPF90 software package (Misztal et al., 2002) was applied. The AI-REML algorithm was used for bivariate models with 2 continuous traits. For runs including one binary trait (nonreturn or disease traits), a Bayesian approach and Gibbs sampling was applied. In total, a chain length of 300,000 samples was generated, and the first 60,000 rounds were discarded as "burn-in." Every 10th sample was saved for the calculation of posterior means and standard deviations. Hence, in total, 24,000 samples were the basis to infer genetic parameters. The number of samples and the length of the burn-in period were determined based on visual inspections for genetic covariances, and on the effective sample size. Misztal et al. (2002) suggested an effective sample size of NS = 30, which was achieved for all runs.

***Repeated Measurement Analysis for Weight Data: Growth Models.*** To identify the most appropriate function for modeling longitudinal weight data, we evaluated 4 nonlinear growth models using the NLMIXED procedure in SAS University Edition (SAS Institute Inc., Cary, NC). These were the logistic growth model (Fekedulegn et al., 1999), the Gompertz growth model (Wellock et al., 2004), the Brody growth model (Fitzhugh, 1976), and the Richards growth model (Fekedulegn et al., 1999). Additionally, 3 linear models with linear, quadratic, and cubic Legendre polynomials (Kirkpatrick et al., 1990) were analyzed using the MIXED procedure in SAS (SAS Institute Inc.). Evaluation criteria were the Akaike's information criterion (**AIC**; Akaike, 1973), the Bayesian information criterion (**BIC**), and residual variances.

***Repeated Measurement Analysis for Weight Data: Estimation of Genetic Parameters.*** An RRM with the time dependent covariate "age in month" was applied to longitudinal BW data. The statistical model [4] was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Qd} + \mathbf{Wm} + \mathbf{Zp} + \mathbf{Sp_m} + \mathbf{e}, \qquad [4]$$

where $\mathbf{y}$ was a vector of observations for longitudinal BW; $\mathbf{b}$ was a vector of fixed effects including herd-year-month, and regressions on age in month using cubic Legendre polynomials nested within birth year; $\mathbf{d}$ was a vector of direct additive-genetic effects for random regression coefficients, which were modeled with Legendre polynomials of order 3; $\mathbf{p}$ was a vector of permanent environmental effects for random regression coefficients, which were modeled with Legendre polynomials of order 3; $\mathbf{m}$ was a vector of random maternal genetic effects; $\mathbf{p_m}$ was a vector of maternal permanent environmental effects; $\mathbf{e}$ was a vector of random residual effects; and $\mathbf{X}$, $\mathbf{Q}$, $\mathbf{W}$, $\mathbf{Z}$, and $\mathbf{S}$ were incidence matrices for $\mathbf{b}$, $\mathbf{d}$, $\mathbf{m}$, $\mathbf{p}$, and $\mathbf{p_m}$, respectively. Heterogeneous residual variances were assumed across the weighing age for the following time intervals: 0, 1 to 4, 5 to 8, 9 to 12, 13 to 16, 17 to 20, and 21 to 24 mo. The following (co)variance structure for random effects was assumed:

$$\mathbf{Var}\begin{bmatrix}\mathbf{u}\\ \mathbf{m}\\ \mathbf{p}\\ \mathbf{p_m}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{G_d}\otimes\mathbf{A} & \mathbf{g_{dm}}\otimes\mathbf{A} & 0 & 0 & 0\\ \mathbf{g'_{dm}}\otimes\mathbf{A} & \mathbf{g_m A} & 0 & 0 & 0\\ 0 & 0 & \mathbf{P}\otimes\mathbf{I_p} & 0 & 0\\ 0 & 0 & 0 & \sigma^2_{\mathbf{p_m}}\mathbf{I_m} & 0\\ 0 & 0 & 0 & 0 & \mathbf{R}\otimes\mathbf{I_n}\end{bmatrix},$$

where $\mathbf{G_d}$ was a 4 × 4 (co)variance matrix of random regression coefficients for the direct additive-genetic effects; $\mathbf{g_m}$ was the maternal-genetic variance; $\mathbf{g_{dm}}$ was the vector of additive genetic covariances between direct and maternal genetic effects; $\mathbf{A}$ was the additive genetic relationship matrix; $\mathbf{P}$ was a 4 × 4 (co)variance matrix of random regression coefficients for permanent environmental effects; $\mathbf{I_p}$ was an identity matrix for p cows; $\boldsymbol{\sigma}^2_{\mathbf{p_m}}$ was the variance of random maternal permanent environmental effects; $\mathbf{I_m}$ was an identity matrix for $m$ dams; $\mathbf{R}$ was a (co)variance matrix for residual effects of dimension 7 × 7 (for the defined 7 time intervals); $\mathbf{I_n}$ was the identity matrix for $n$ observations, and ⊗ was the direct matrix product. Also for the RRM analyses, Gibbs sampling as implemented in BLUP90 (Misztal et al., 2002) was applied, again using a chain length of 300,000 samples, and considering a burn-in period of 60,000 rounds. The thinning interval was set to 10.

## RESULTS AND DISCUSSION

### Genetic Parameters for Single BW Measurements

Direct, maternal, and total heritabilities for CABW and IBW are given in Table 4. The direct heritability of 0.47 for CABW was in line with the moderate to high direct heritabilities as reported in previous studies (Table 1), for example, a direct heritability of 0.53 based on weight records from 486 Holstein calves kept in one experimental herd (Coffey et al., 2006). McCorquodale et al. (2013) analyzed weight data from 1,588 Holstein calves. The direct heritability including a recording period from 0 to 8 d of age was 0.44. The maternal heritability of 0.19 from our study was larger compared with previous estimates in dairy cattle [e.g., Everett and Magee (1965); Johanson et al. (2011)]. Fisher and Williams (1978) also found a quite large maternal genetic heritability of 0.26 using data from 1,552 Holstein calves. In Nellore beef cattle, heritabilities were 0.32 and 0.10 for direct and maternal effects, respectively (Chud et al., 2014). In the Charolais, Limousin, Blonde d′Aquitaine, and Maine-Anjou breeds, the direct heritabilities ranged from 0.28 to 0.38, and the corresponding maternal heritabilities were in the range from 0.08 to 0.10 (Phocas and Laloë, 2004). Apparently, in beef as well as in dairy cattle populations, direct heritabilities were significantly larger than the maternal component.

Direct and maternal heritabilities for IBW were significantly smaller compared with estimates for CABW (Table 4). Coffey et al. (2006) also found a decrease of direct BW heritabilities during the time span from birth to weaning age. The genetic part of the total heritability included the direct additive genetic variance for the calves, the maternal genetic variance, and the

**Table 4**. Direct ($h_d^2$), maternal ($h_m^2$), and total ($h_t^2$) heritabilities and genetic correlations between direct and maternal genetic effects ($r_{dm}$) for calf birth weight (CABW) and BW at first insemination (IBW)

| Weight | $h_d^2$ | SE | $h_m^2$ | SE | $h_t^2$ | SE | $r_{dm}$ | SE |
|---|---|---|---|---|---|---|---|---|
| CABW | 0.47 | 0.02 | 0.19 | 0.01 | 0.39 | 0.01 | −0.39 | 0.02 |
| IBW | 0.20 | 0.01 | 0.06 | 0.00 | 0.19 | 0.01 | −0.24 | 0.03 |

covariance between direct and maternal genetic effects. Therefore, given the negative covariance between the 2 genetic sources, the total heritability or realized heritability of mass selection (Willham, 1972) was lower than the direct heritability. The genetic correlation between direct effects of CABW and IBW was 0.31, indicating the changing genetic background of BW with aging.

The genetic correlations between direct and maternal genetic effects were −0.39 for CABW and −0.24 for IBW. Accuracy of genetic correlation estimates between direct and maternal genetic effects increases with an increasing number of dams with phenotypes (Heydarpour et al., 2008). In our data set, the 57,868 calves with records for CABW were offspring of 43,714 dams, and the 9,462 heifers with records for IBW were offspring of 8,729 dams. However, only 13,800 dams were phenotyped for CABW, and only 731 dams had records for IBW, indicating 68.43 and 91.62% of dams with missing BW records, respectively. Heydarpour et al. (2008) recommended a proportion of dams with missing records smaller than 50%, to avoid biased estimates for maternal genetic and maternal permanent environmental variances, and for the direct-maternal covariance component. In consequence, we additionally performed runs based on a reduced data set including only the dams with phenotypes. For this specific data set, the maternal heritability for CABW was 0.22, and 0.12 for IBW. The direct-maternal genetic correlation was −0.42 for CABW, and −0.26 for IBW. Hence, the results from the analysis as suggested by Heydarpour et al. (2008) confirmed the estimates as presented in Table 4. The negative genetic correlation between direct and maternal effects was identified in several previous beef cattle analyses, indicating incompatibility between genes increasing an animal's BW and genes contributing to improved maternal performance of a cow (Garrick et al., 1989). Hence, similar genetic or physiological mechanisms seem to exist in dairy cattle. In this regard, Lee (2001) stated that a "biological explanation of genetic antagonism between direct and maternal genetic effects is currently unavailable." From a biological perspective, Bauman and Currie (1980) addressed nutrient competition for either growth or milk yield traits, and Bijma (2006) raised questions addressing genetic modeling aspects. However, also when accounting in statistical models for environmental covariances between dam and offspring records, the antagonistic relationship between direct and maternal effects still existed (Bijma, 2006).

### *Correlations Between BW and Test-Day Traits*

Genetic and phenotypic correlations between CABW and IBW with test-day traits are given in Table 5. Heritabilities were moderate for production traits, and were low for SCS. The heritabilities for the same trait were identical from 2 runs including either CABW or IBW. In agreement with Mahmoud et al. (2017), heritabilities in same traits were larger at the second official test-day compared with estimates from the first official test-day. Generally, genetic and phenotypic correlations between CABW with productivity and SCS were close to zero. Throughout negative genetic correlations (but also on a very low level) were detected between CABW with FP and with FPR from both test-days. As an indicator for a cow's energy status (Friggens et al., 2007), a high FPR indicates negative energy balance and a quick mobilization of body fat depots. Therefore, the negative genetic correlations between CABW and FPR suggest that animals with large BW early in life tend to prevent energy deficiency early in lactation. However, it is a long time span from the calf toward the milking cow stage. Similarly, only weak associations were identified between the calf health status and milking cow production traits (Mahmoud et al., 2017). The time point for measuring IBW is closer to the lactation stage, and accordingly, a stronger effect of IBW on cow test-day traits was identified (Table 5). The highest genetic correlations were found between IBW with protein yield (i.e., 0.22 with PY1 and 0.24 with PY2). Also, genetic correlations between IBW with MY1 and MY2 were positive, indicating that higher IBW marginally increased milk production at first and second test-days. Close to zero genetic correlations were found between averaged BW records with milk production traits from multiparous Holstein-Friesian cows (Berry et al., 2003a). Veerkamp et al. (2000) correlated BW and production traits from the same lactation, but also from overlapping periods for both trait categories; genetic associations had a minor effect.

**Table 5**. Heritabilities for test-day traits, and genetic ($r_g$) and phenotypic ($r_p$) correlations between test-day traits with calf birth weight (CABW) and BW at first insemination (IBW)

| Test-day trait[1] | CABW with test-day traits | | | | | IBW with test-day traits | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $h^2$ | SE | $r_g$ | SE | $r_p$ | $h^2$ | SE | $r_g$ | SE | $r_p$ |
| MY1 | 0.16 | 0.001 | 0.05 | 0.003 | −0.01 | 0.16 | 0.001 | 0.11 | 0.003 | 0.08 |
| MY2 | 0.25 | 0.001 | 0.05 | 0.003 | −0.01 | 0.25 | 0.001 | 0.16 | 0.003 | 0.11 |
| FP1 | 0.15 | 0.001 | −0.12 | 0.003 | 0.01 | 0.15 | 0.001 | −0.06 | 0.003 | 0.07 |
| FP2 | 0.29 | 0.002 | −0.06 | 0.003 | 0.01 | 0.29 | 0.002 | −0.02 | 0.003 | 0.08 |
| FY1 | 0.19 | 0.001 | −0.06 | 0.003 | −0.00 | 0.19 | 0.001 | 0.06 | 0.003 | 0.13 |
| FY2 | 0.21 | 0.001 | −0.05 | 0.003 | −0.00 | 0.21 | 0.001 | 0.11 | 0.003 | 0.17 |
| PP1 | 0.16 | 0.001 | −0.05 | 0.003 | 0.00 | 0.16 | 0.001 | 0.17 | 0.003 | 0.01 |
| PP2 | 0.37 | 0.002 | 0.00 | 0.003 | 0.00 | 0.36 | 0.002 | 0.10 | 0.003 | −0.01 |
| PY1 | 0.15 | 0.001 | 0.04 | 0.003 | −0.01 | 0.15 | 0.001 | 0.22 | 0.003 | 0.09 |
| PY2 | 0.18 | 0.001 | 0.05 | 0.003 | −0.01 | 0.19 | 0.001 | 0.24 | 0.003 | 0.12 |
| FPR1 | 0.15 | 0.001 | −0.10 | 0.003 | 0.01 | 0.15 | 0.001 | −0.15 | 0.003 | 0.07 |
| FPR2 | 0.21 | 0.001 | −0.06 | 0.003 | 0.01 | 0.22 | 0.001 | −0.09 | 0.003 | 0.09 |
| SCS1 | 0.09 | 0.001 | −0.01 | 0.003 | 0.01 | 0.09 | 0.001 | 0.11 | 0.003 | −0.00 |
| SCS2 | 0.09 | 0.001 | 0.05 | 0.003 | 0.02 | 0.09 | 0.001 | 0.09 | 0.003 | 0.00 |

[1]MY = milk yield; FP = fat percentage; PP = protein percentage; FY = fat yield; PY = protein yield; FPR = fat-to-protein ratio. 1 indicates the observation from the first test-day after calving; 2 indicates the observation from the second test-day after calving.

### *Correlations Between BW and Nonreturn Rates*

As a female fertility trait, heritabilities for NRR56 and NRR90 were consistently small in all bivariate runs with either CABW or IBW (Table 6). The low heritabilities for nonreturn rates correspond with large-scale studies conducted in Holstein cows (Weigel and Rekaya, 2000) or in Norwegian Red (Andersen-Ranberg et al., 2005). As found for the cow test-day traits, small phenotypic correlations were identified between BW measurements and heifer fertility traits (Table 6). Genetic correlations between nonreturn rates with CABW were slightly negative (−0.10 for NR56 and −0.11 for NR90), but positive between nonreturn rates and IBW (0.18; Table 6). Genetically, increasing IBW benefitted nonreturn rates of heifers, indicating the positive associations between improved BW development around insemination time and female fertility. Phenotypic correlations between either CABW or IBW with nonreturn rates were very close to zero (Table 6). Only a limited number of studies focused on associations between BW and nonreturn rates in dairy cattle. Veerkamp et al. (2001) used the BW indicator BCS, and found negative genetic correlations between BCS with the calving interval, and with the time span from calving to first service in first-lactation cows. The genetic correlation between BCS and NRR56 in first lactation was −0.10 (Banos et al., 2004), and −0.11 between BW in the first week after calving and commencement of first luteal activity (Veerkamp et al., 2000). From a management perspective, the "close to zero" phenotypic correlations with female fertility traits suggest an intermediate optimum for BW or BW indicators (e.g., BCS) around insemination time. Genetically, Abdallah and McDaniel (2000) reported that heavier cows from 6 experimental herds conceived earlier than smaller cows.

### *Correlations Between BW and Health Traits*

Heritabilities for disease traits as well as genetic and phenotypic correlations between health traits with CABW and IBW are shown in Table 7. Heritabilities for health traits are in agreement with estimates from previous studies, that is, with Mahmoud et al. (2017) for cow respiratory diseases, with Harder et al. (2006) for metabolic disorders, with König et al. (2005) or Ger-

**Table 6**. Posterior means and posterior SD of the Gibbs samples for heritabilities ($h^2$) of nonreturn rate after 56 (NRR56) and 90 d (NRR90), and for genetic ($r_g$) and phenotypic ($r_p$) correlations between nonreturn rates and calf birth weight (CABW) and BW at first insemination (IBW)

| Heifer fertility trait | CABW with nonreturn rate | | | | | IBW with nonreturn rate | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $h^2$ | SD | $r_g$ | SD | $r_p$ | $h^2$ | SD | $r_g$ | SD | $r_p$ |
| NRR56 | 0.03 | 0.01 | −0.10 | 0.08 | −0.01 | 0.02 | 0.01 | 0.18 | 0.17 | −0.03 |
| NRR90 | 0.03 | 0.01 | −0.11 | 0.08 | −0.00 | 0.01 | 0.01 | 0.18 | 0.18 | −0.03 |

**Table 7**. Posterior means and posterior SD of the Gibbs samples for heritabilities ($h^2$) of health traits, and for genetic ($r_g$) and phenotypic ($r_p$) correlations between disease traits and calf birth weight (CABW) and BW at first insemination (IBW)

| | CABW with health traits | | | | | IBW with health traits | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Disease trait[1] | $h^2$ | SD | $r_g$ | SD | $r_p$ | $h^2$ | SD | $r_g$ | SD | $r_p$ |
| GDS | 0.26 | 0.01 | 0.22 | 0.04 | 0.04 | 0.26 | 0.01 | 0.03 | 0.09 | 0.05 |
| DIA | 0.13 | 0.02 | 0.09 | 0.08 | 0.04 | 0.15 | 0.02 | −0.03 | 0.14 | −0.02 |
| RD | 0.03 | 0.01 | 0.22 | 0.18 | 0.02 | 0.07 | 0.02 | −0.24 | 0.30 | 0.01 |
| MAST | 0.13 | 0.01 | 0.15 | 0.05 | 0.02 | 0.13 | 0.01 | 0.02 | 0.10 | 0.07 |
| CL | 0.13 | 0.01 | 0.21 | 0.05 | 0.05 | 0.14 | 0.01 | 0.11 | 0.09 | 0.09 |
| FF | 0.12 | 0.013 | 0.24 | 0.05 | 0.04 | 0.13 | 0.012 | 0.11 | 0.10 | 0.04 |
| MET | 0.12 | 0.033 | −0.09 | 0.11 | 0.00 | 0.14 | 0.031 | −0.04 | 0.17 | 0.06 |

[1]GDS = general disease status; DIA = diarrhea; RD = respiratory disease; MAST = mastitis; CL = claw disorders; FF = female fertility disorders; MET = metabolic disorders.

nand et al. (2012) for claw disorders, and with Zwald et al. (2006) for clinical mastitis. Genetic correlations between CABW and health traits were larger than the corresponding correlations with test-day traits or with nonreturn rates, but also in a narrow range from −0.09 to 0.22. Genetic correlations were positive and larger than 0.10 between CABW with GDS, MAST, CL, and FF. The positive correlation indicates, from a genetic perspective, that heavier calves have a higher risk for disease occurrence later in life. McCorquodale et al. (2013) stated that calves with higher CABW had less disease early in life, along with improved disease resistance during aging. In contrast, Mahmoud et al. (2017) found improved disease resistance after calving for cows with respiratory and digestive infections during calfhood, independent from growth development. Genetic correlation estimates were even weaker between IBW and cow disease traits. The only obvious difference when comparing CABW and IBW correlations was identified for RD: the genetic correlation between CABW and RD was positive (0.22), but negative between IBW and RD (−0.24). Hence, alterations of genetic covariances between BW development and RD suggest deeper analyses in this regard, from a genetic-statistical perspective (e.g., application of RRM), as well as from molecular applications (e.g., studies on gene expressions). The negative correlations between both BW measurements CABW and IBW with MET indicates that animals with more BW or better body development are less susceptible to metabolic disorders (Frigo et al., 2010). Further BW–disease genetic associations were studied in lactating cows. Pérez-Cabal and Charfeddine (2016) reported positive correlations (i.e., antagonistic associations) between BW with sole ulcer, white line disease, and overall definitions of claw disorders. Frigo et al. (2010) found a slightly positive genetic correlation of 0.15 between BW at DIM 10 and mastitis using data from 2 experimental dairy herds.

### *Genetic Parameters for Longitudinal BW*

The model evaluation criteria AIC, BIC, and residual variances for 4 nonlinear models and Legendre polynomials 1 to 3 orders are compared in Table 8. Based on AIC, the Gompertz growth model gave the best fit. Residual variances and BIC indicated model superiority for Legendre polynomials of third order. Köhn et al. (2007) also suggested modeling of growth curves of the Göttingen minipig using third-order polynomials. Goldberg and Ravagnolo (2015) compared 5 nonlinear models and concluded that the Richards model gave best predictions of weights from birth to maturity for Angus cows kept in pasture-based production systems. Random regression models using cubic Legendre polynomials and cubic regressions were suggested for the description of growth curves in Nellore cattle (Nobre et al., 2003) and in dairy cattle (Brotherstone et al., 2007), respectively.

Due to the results from growth curve evaluation, time-dependent covariates in genetic studies with RRM were modeled using Legendre polynomials of order 3. Total, direct genetic, and maternal genetic heritabilities for longitudinal BW by age in months are depicted in Figure 1. The direct and total heritabilities overlapped,

**Table 8**. Akaike information criterion (AIC), Bayesian information criterion (BIC), and residual variances for longitudinal BW analyses (values in bold represent the lowest value for the respective evaluation criterion)

| Model | AIC | BIC | Residual |
|---|---|---|---|
| Logistic | 320,503 | 320,536 | 1,157 |
| Gompertz | **316,269** | 316,302 | **1,015** |
| Brody | 316,968 | 317,001 | 1,037 |
| Richards | 338,014 | 338,056 | 1,843 |
| Legendre polynomial 1 | 317,531 | 317,537 | 1,055 |
| Legendre polynomial 2 | 316,942 | 316,949 | 1,036 |
| Legendre polynomial 3 | 316,294 | **316,300** | **1,015** |

apart from small differences early in life (0 to 4 mo). Identical estimates from 5 to 24 mo were due to the extremely small covariance between direct and maternal genetic effects. The direct and total heritabilities were larger than 0.49 at birth, decreased to 0.26 at age mo 1, and increased gradually to 0.57 at age mo 4. A gradually increase of direct and total heritabilities was observed between mo 5 and 24, with the largest estimate of 0.83 at the end of the continuous age scale. However, extremely large genetic variances and heritabilities at the peripheries of environmental or time scales were observed in previous RRM applications (Berry et al., 2003b; Strabel et al., 2005), especially in small data sets. Direct BW heritabilities from this study are in agreement with estimates from RRM considering a time span from birth to the age of 33 mo (Brotherstone et al., 2007). Only during the period between 20 to 24 mo, direct heritabilities from our study were significantly larger.

As expected, the maternal heritability for BW gradually decreased from birth to an age of 5 mo, and was zero afterward (Nobre et al., 2003). The direct, maternal, and total heritabilities for birth weight estimated from RRM in the present study were 0.58, 0.16, and 0.49, respectively. The direct heritability was slightly larger than the corresponding heritability estimated from the single-trait animal model for CABW, but the maternal heritability was slightly lower. Insemination age for the heifers ranged from 11 to 26 mo, and the direct and total heritabilities during this period were always larger than 0.37. Those heritabilities were larger than the direct and maternal heritabilities for IBW from the single-trait animal model.

Genetic correlations between BW at birth with BW from remaining age points gradually decreased from 1.00 to 0.12 at an age of 4 mo (Figure 2). Large genetic correlations for neighboring test-days, but a substantial decrease for test-days in greater distance, is well known for longitudinal production records within lactation (e.g., Strabel and Misztal, 1999). The physiological background might be that different genes are "switched on or off" with aging or with DIM. The genetic correlation between BW at birth and at 24 mo was even negative (−0.20). Hence, BW from age points in great distance are genetically different traits. Accordingly, Brotherstone et al. (2007) reported decreasing genetic correlations with an increasing interval between birth and weight date. Genetic correlations between BW from different time points were larger in the study by Coffey et al. (2006). They found a genetic correlation of 0.79 between birth weight and weaning weight in a Holstein-Friesian dairy cattle population. The respective genetic correlation in our study, considering a similar time period, was only 0.20.

Genetic correlations between direct and maternal genetic effects at the same age were negative throughout

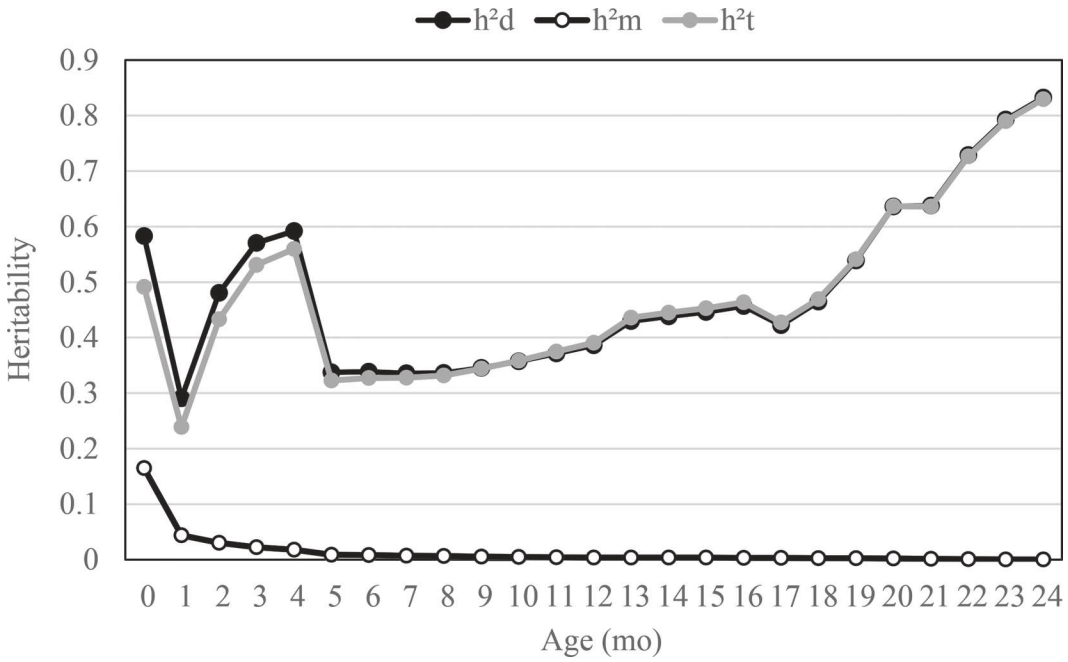


**Figure 1**. Posterior means of the Gibbs samples for direct ($h^2$d), maternal ($h^2$m), and total heritabilities ($h^2$t) for longitudinal BW. Posterior SD for direct heritabilities ranged from 0.02 to 0.06, for maternal heritabilities from 0.00 to 0.04, and for total heritabilities from 0.01 to 0.04.

from birth to the age of 10 mo, and from the age of 20 to 24 mo. The correlation coefficient of −0.39 at d 0 was exactly the same value as estimated with an animal model with maternal genetic effects (model 1, results in Table 4). The negative genetic correlations between direct and maternal effects for birth weight were also found in other dairy cattle populations (Hansen et al., 2004; Johanson et al., 2011), as well as in beef cattle (Chud et al., 2014). Boujenane et al. (2015) also stated that those correlations were negative for birth weight, and for BW at 90 and 135 d in D'man sheep. The direct genetic effect was slightly positive correlated with the maternal genetic effect from mo 11 to 19. In contrast, a negative correlation of −0.24 was found for IBW and model [1] applications. However, for BW later in life, the maternal genetic component was almost zero, implying that minor changes in covariances can have a major effect on correlation coefficients.

## CONCLUSIONS

As found in beef cattle studies, BW recorded from Holstein dairy calves (trait: CABW) and heifers (trait: IBW) had a direct genetic and a maternal genetic component. The direct heritability for CABW estimated from single-trait animal models with maternal genetic effects was 0.47, and 0.20 for IBW. Comparable direct heritabilities were obtained from RRM for a longitudinal BW data structure. In addition, an antagonistic relationship between direct genetic and maternal genetic effects around birth date was identified, with −0.39 for CABW from single trait as well as repeated measurement analyses. The maternal genetic heritability was 0.19 for CABW, but close to zero later in life, especially in RRM applications. Genetic and phenotypic correlations between CABW or IBW with either cow test-day traits and cow health traits, or with female fertility traits from heifers, were close to zero. However, apart from MET, a general tendency was observed for increasing disease susceptibilities in early lactation for heavier calves.

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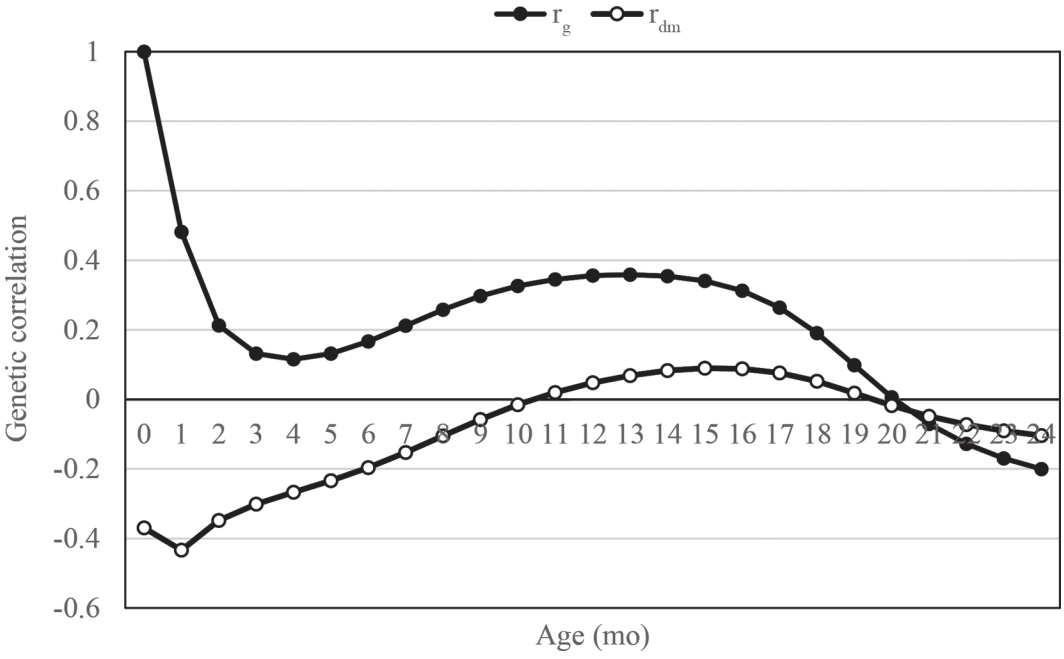


**Figure 2**. Posterior means of the Gibbs samples for the genetic correlations between calf birth weight and BW from the remaining ages ($r_g$), and for the correlations between direct and maternal effects across BW ages ($r_{dm}$). Posterior SD for $r_g$ ranged from 0.00 to 0.06, and for $r_{dm}$ from 0.09 to 0.12.

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## 2.5. Original research paper 5

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# Genome-wide associations and detection of potential candidate genes for direct genetic and maternal genetic effects influencing dairy cattle body weight at different ages

Tong Yin and Sven König*

## Abstract

**Background:** Body weight (BW) at different ages are of increasing importance in dairy cattle breeding schemes, because of their strong correlation with energy efficiency traits, and their impact on cow health, longevity and farm economy. In total, 15,921 dairy cattle from 56 large-scale test-herds with BW records were genotyped for 45,613 single nucleotide polymorphisms (SNPs). This dataset was used for genome-wide association studies (GWAS), in order to localize potential candidate genes for direct and maternal genetic effects on BW recorded at birth (BW0), at 2 to 3 months of age (BW23), and at 13 to 14 months of age (BW1314).

**Results:** The first 20 principal components (PC) of the genomic relationship matrix (**G**) grouped the genotyped cattle into three clusters. In the statistical models used for GWAS, correction for population structure was done by including polygenic effects with various genetic similarity matrices, such as the pedigree-based relationship matrix (**A**), the **G**-matrix, the reduced **G**-matrix LOCO (i.e. exclusion of the chromosome on which the candidate SNP is located), and LOCO plus chromosome-wide PC. Inflation factors for direct genetic effects using **A** and LOCO were larger than 1.17. For **G** and LOCO plus chromosome-wide PC, inflation factors were very close to 1.0. According to Bonferroni correction, ten, two and seven significant SNPs were detected for the direct genetic effect on BW0, BW23, and BW1314, respectively. Seventy-six candidate genes contributed to direct genetic effects on BW with four involved in growth and developmental processes: *FGF6*, *FGF23*, *TNNT3*, and *OMD*. For maternal genetic effects on BW0, only three significant SNPs (according to Bonferroni correction), and four potential candidate genes, were identified. The most significant SNP on chromosome 19 explained only 0.14% of the maternal de-regressed proof variance for BW0.

**Conclusions:** For correction of population structure in GWAS, we suggest a statistical model that considers LOCO plus chromosome-wide PC. Regarding direct genetic effects, several SNPs had a significant effect on BW at different ages, and only two SNPs on chromosome 5 had a significant effect on all three BW traits. Thus, different potential candidate genes regulate BW at different ages. Maternal genetic effects followed an infinitesimal model.

## Background

Some countries with pasture-based production systems consider dairy cow live weight in overall breeding goals or in selection indices [1, 2]. Positive genetic correlations of body weight (BW) with milk yield and protein yield have been reported [3–5]. Feed efficiency reflects the ability of dairy cows to produce more milk for a given feed consumption [6]. Different traits are defined to measure feed efficiency, e.g. the ratio of milk to body weight, feed intake, residual feed intake [7], and feed saved [8]. Most of these definitions imply that BW or changes in BW are taken into account. Moreover, dry matter intake and energy balances are favourably correlated with BW [3]. In addition, BW influences dairy cow fertility and health. For example, survival of new-born calves and calving ease are moderately correlated with birth weight

*Correspondence: Sven.Koenig@agrar.uni-giessen.de
Institute of Animal Breeding and Genetics, Justus-Liebig-University Gießen, Ludwigstr. 21b, 35390 Giessen, Germany

of calves and BW of cows [9]. Berry et al. [5] reported that heavier cows had a shorter interval between calving and first service, but conception rates decreased with increasing BW. In contrast, in heifers, increasing BW was associated with improved non-return rates after 56 and 90 days [4]. Hence, we hypothesize that different genes are involved in BW at different ages, as indicated in quantitative genetic studies via random regression models [4].

On the genomic scale, GWAS for BW or BW indicators have considered only one time point per animal [10–12]. Zhang et al. [12] analysed longitudinal BW records in cattle at 6, 12, 18 and 24 months of age, but BW was predicted from measurements for heart girth and hip height. The aforementioned publications focussed only on the estimation of direct additive genetic effects on BW. However, especially in early life, BW should be separated into direct genetic and maternal genetic effects [13]. Dams with high breeding values for maternal ability provide an improved nourishing environment, with an associated positive impact on survival rates and birth weight in offspring. For a deeper understanding of the mechanisms between direct and maternal effects, it is imperative to detect the functional segments of the genome that contribute to maternal genetic effects on BW, and to study direct-maternal associations on the genomic scale. To date, only a few studies [14–16] have addressed such topics.

The power of GWAS contributes to the detection of significant markers, and, furthermore, has an impact on the identification of associated potential candidate genes. Linkage disequilibrium (LD) is one of the parameters that affects the power of GWAS. The use of a dense 50 K single nucleotide polymorphism (SNP) chip implies that it contains markers that are closely located to the functional mutation and contribute to acceptable LD between markers and causal loci [17]. Body weight is a trait with a moderate to high pedigree-based heritability [4, 5, 18], which is favourable for the detection of QTL. Furthermore, currently, the trend is to use large numbers of female observations for the estimation of SNP effects, which contributes to an increasing number of phenotypic records for GWAS [19], with a positive impact on the statistical power for the detection of SNP effects. Non-causative rare alleles with high frequencies in large half-sib daughter groups might contribute to false positive signals in GWAS. Usually, the first principal components and similarity matrices can be considered in statistical modelling to correct for population stratification [20]. In dairy cattle breeding, deep pedigree information is available, which enables the use of mixed models for GWAS with random polygenic effects based on pedigree [21] or on genomic relationship matrices [22].

Consequently, the objectives of our study were: (1) to perform GWAS using phenotypes and de-regressed proofs for direct genetic and maternal genetic effects on BW at different ages; (2) to correct for population stratification in GWAS when using pedigree-based or genomic relationship matrices, or a combination of relationship matrices with principal components; (3) to infer (co)variance components for/between direct genetic and maternal genetic effects on different scales (pedigree-based genetic parameters, whole genome, and single chromosomes); and (4) to identify associated potential candidate genes for direct genetic and maternal genetic effects.

## Methods

### Phenotype data

Body weight records at birth (BW0), 2 to 3 months of age (BW23), and 13 to 14 months of age (BW1314) were available for 250,173, 42,632 and 54,768 female animals, respectively. The number of animals with phenotypic records at all three age intervals was 15,234. Animals were born between 2004 and 2016, and kept in 56 large-scale dairy cattle test-herds, which were located in the German federal states of Mecklenburg-Westpommerania and Berlin-Brandenburg. For the 250,173 calves, the gestation length of their dams ranged from 265 to 295 days (average: 279.4 days). For BW0, we discarded birth weights above 60 kg or below 20 kg. For the detection of outlier data for BW23 and BW1314, we followed the approach by Yin et al. [4] and calculated studentized residuals and corresponding Bonferroni $p$ values (using the outlier test function in the R package "car" [23]). Records were excluded from further analyses when $p$ values were lower than 0.05 or higher than 0.95. The pedigree file included 411,943 animals, born between 1948 and 2016.

### Genotype data

Among the Holstein cattle with BW records, 13,827 calves with BW0 records, 4246 calves with BW23 records, and 7920 heifers with BW1314 records, were genotyped. Genotyping was performed using the Illumina Bovine 50 K SNP BeadChip V2 (4120 animals), or the Illumina Bovine Eurogenomics 10 K low-density chip (11,801 animals). Animals with low-density genotypes were imputed to the 50 K chip (according to the routine procedure for official national genetic evaluations [24]). Finally, for all the genotyped cattle, 45,613 SNPs were available that had a call rate higher than 95%, a minor allele frequency higher than 0.01, and did not deviate significantly from Hardy–Weinberg equilibrium ($p > 0.001$). Only SNPs located on *Bos taurus* autosomes (BTA) were considered. Furthermore, we discarded animals with more than 95% identical genotypes. Quality control of SNP data was done by using the GenABEL package in R [25]. In order to verify the impact of LD between SNPs

on the inflation factors in GWAS, we applied the indep-pairwise option in PLINK [26]. We eliminated one SNP from pairs of SNPs that had a LD coefficient ($r^2$) higher than 0.25 [27]. The remaining SNPs after this elimination procedure were defined as pruned SNPs. The numbers of animals, numbers of the full SNPs, and numbers of pruned SNPs, are in Table 1.

### Population stratification

The genomic relationship matrix **G** was constructed as in [28] based on the full SNP dataset, and then used for principal component analysis, in order to visualise possible population stratification for the 15,921 genotyped animals. The software package GCTA [29] generated the first 20 principal components (PC). Then, k-means clustering was applied by including the first 10 PC, because the remaining 10 PC were not informative and overloaded k-means clustering.

### Statistical models

#### *Pedigree-based (co)variance components and breeding values*

A multiple-trait animal model was defined, in order to infer the genetic components and to estimate breeding values for direct genetic and maternal genetic effects. In this regard, we applied restricted maximum likelihood (REML) via AIREMLf90 from the BLUPF90 software package [30]. The statistical Model 1 for the three BW traits (BW0, BW23, BW1314) in matrix notation was:

**Table 1 Number of animals and SNPs for the genome-wide association studies**

| Trait | Dependent variable | #animals[a] | #animals[b] | #markers[c] | #markers[d] |
|---|---|---|---|---|---|
| BW0 | Phen | 13,827 | 13,714 | 42,468 | 11,955 |
| | dDRP | 15,921 | 14,121 | 42,465 | 11,954 |
| | mDRP | 16,455 | 16,022 | 42,540 | |
| BW23 | Phen | 4246 | 4219 | 42,388 | 11,908 |
| | dDRP | 15,921 | 8017 | 42,421 | 11,933 |
| | mDRP | 16,455 | 6803 | 42,498 | |
| BW1314 | Phen | 7920 | 7874 | 42,443 | 11,943 |
| | dDRP | 15,921 | 7874 | 42,443 | 11,943 |
| | mDRP | 16,455 | 6996 | 42,503 | |

BW0: body weight recorded at birth; BW23: body weight recorded at 2 to 3 months of age; BW1314: body weight recorded at 13 to 14 months of age; dDRP: de-regressed proofs for the direct genetic effect; mDRP: de-regressed proofs for the maternal genetic effect

[a] Number of cows with genotypes

[b] Number of cows with genotypes after quality control

[c] Number of markers after quality control

[d] Number of markers after pruning

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Wm} + \mathbf{Sp_m} + \mathbf{e}, \quad (1)$$

where **y** is a vector of phenotypes for BW0, BW23, and BW1314 from 250,173, 42,632 and 54,768 female animals, respectively; **b** is a vector of fixed effects, including herd, birth year, birth month, and the covariate (linear regression) gestation length for BW0, and age (in days) of the calves/heifers for BW23 and BW1314; **u** is a vector of direct additive-genetic effects, with $\mathbf{u} \sim N(0, \mathbf{A}\sigma_u^2)$, where **A** is the pedigree-based relationship matrix and $\sigma_u^2$ is the direct-genetic variance; **m** is a vector of random maternal-genetic effects, with $\mathbf{m} \sim N(0, \mathbf{A}\sigma_m^2)$, where $\sigma_m^2$ is the maternal-genetic variance; $\mathbf{p_m}$ is a vector of random maternal permanent environmental effects; **e** is a vector of random residual effects; and **X**, **Z**, **W**, and **S** are incidence matrices for **b**, **u**, **m**, and $\mathbf{p_m}$, respectively.

Estimated breeding values from Model 1 for direct genetic and maternal genetic effects were used to calculate de-regressed proofs (DRP) for the direct genetic (dDRP) and for the maternal genetic component (mDRP), respectively, according to Garrick et al. [31]. Only the animals with a DRP weight greater than 0.2 were considered in ongoing GWAS (see Model 3). The number of DRP records for direct genetic and maternal genetic effects is in Table 1. Since animal models generate breeding values for all the animals from the pedigree database, all these animals, including the phenotyped and non-phenotyped animals, were considered for DRP calculations, which means that an increased number of genotyped animals for DRP is available.

#### *Genomic heritabilities and correlations*

Variance components and correlations for the three BW traits explained by SNPs on all the chromosomes were estimated via genomic REML (GREML), as implemented in GCTA [29]. Model 2 was defined as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e}, \quad (2)$$

where, **y** is a vector of phenotypes for BW0, BW23, and BW1314, and **b** is a vector of fixed effects including the same effects as specified in Model 1. The variance for additive genetic effects **u** was equal to $\mathbf{G}\sigma_u^2$, with G representing the genomic relationship, and $\sigma_u^2$ representing the variance explained by SNPs from the full dataset. For the estimation of covariance components in bivariate models, GCTA requires that the fixed effects are the same for both traits. Hence, we ran bivariate models for pairwise combinations of pre-corrected phenotypes for BW0, BW23, and BW1314. The pre-corrected phenotype for a specific genotyped animal was the sum of the estimated direct breeding value, the maternal breeding value, the maternal environmental effect, and the residual (i.e. output from Model 1).

#### *Genome-wide association studies*

The software package GCTA [29] was also used to estimate SNP effects via linear mixed models with a random polygenic effect. The statistical Model 3 for single marker regression analysis was:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Wg} + \mathbf{Zu} + \mathbf{e}, \quad (3)$$

where **y** is a vector of phenotypes, dDRP or mDRP for BW0, BW23, and BW1314; **b** is a vector of fixed effects including the same effects as specified in Model 1 for phenotypes as dependent variables, but for DRP, **b** only considered the overall mean effect; **g** is the vector for SNP effects; **u** is a vector of polygenic effects with a variance–covariance structure of $\mathbf{u} \sim N(0, \mathbf{K}\sigma_u^2)$, where **K** is the genetic similarity matrix between individuals, and $\sigma_u^2$ is the polygenic variance; **e** is a vector of random residual effects with $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$; and **X**, **W**, and **Z** are incidence matrices for **b**, **g**, and **u**, respectively. According to the Bonferroni correction, the defined GWAS significant threshold was 0.05/N, where N refers to the number of SNPs. In addition to the Bonferroni correction, a less conservative adjusted *p* value, based on false discovery rate (FDR), was calculated for each SNP [32]. The threshold for FDR significance was 0.05.

The genetic similarity matrix **K** was constructed with different information sources. First, we created **K** based on the pedigree relationship matrix **A**, as generated from AIREMLF90. Second, the construction of **K** was based on the genomic relationship matrix **G**. Due to possible undesired effects of SNP double-counting [33], alternative **G**-matrices excluded all SNPs from the chromosome on which the candidate SNP is located. This strategy is defined as "leave-one-chromosome-out" (LOCO) [34]. Since the length of bovine chromosomes is not constant, many SNPs on the large chromosomes are excluded. Hence, SNPs located on the large chromosomes BTA1 to 11 (these chromosomes contain more than 1500 SNPs) were separated into two segments per chromosome. The modified LOCO approach (LOCO_SEG40, i.e. the 22 segments from chromosomes 1–11 plus the remaining 18 chromosomes) constructed **G**-matrices using all SNPs, except those from the respective chromosome segment (for BTA1 to BTA11), or excluding all SNPs from the whole chromosome (for BTA12 to BTA30). In addition, chromosomes were separated into smaller segments according to the number of SNPs with (a) segments including 90–100 SNPs (a total of 441 segments = LOCO_SEG441), and (b) segments including 47–50 SNPs (a total of 864 segments = LOCO_SEG864). In order to account for the loss in similarity due to the deleted chromosome in LOCO, the first 20 PC were included as covariates (LOCO + PC20). However, consideration of 20 PC combined with the LOCO **G**-matrix implies partial overlap of genomic information. Hence, as a further alternative, we focussed on principal component analyses for the **G**-matrix from each chromosome, and the first 3, 10 or 20 PC were included as covariates (LOCO + CHR_PC3, LOCO + CHR_PC10, and LOCO + CHR_PC20, respectively). All the similarity matrices (**G**-matrix, LOCO **G**-matrix, and **G**-matrix from each chromosome) as mentioned above were constructed based on the full SNP dataset. An additional LOCO scenario using the pruned SNPs (LOCO_prune) was considered, in order to test the effect of LD between SNPs on inflation.

We used the inflation factor ($\lambda$) as evaluation criterion for the different approaches, which was calculated based on the $\chi_i^2$ statistic for the *i*-th SNP:

$$\widehat{\lambda} = \frac{Median(\chi_i^2)}{0.4549}.$$

The expected inflation factor of value 1 indicates sufficient correction for population stratification. A value above 1.05 indicates inflation in the sample [35], and thus that the detected genome-wide associations might be false positives.

#### *Chromosome-wide genomic parameters*

Genetic variances for each chromosome were estimated via GREML using the full SNP dataset, and applying GCTA [29]. The univariate Model 4 was:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{u_i} + \mathbf{Z}_2\mathbf{u_{all\_without\_i}} + \mathbf{e}, \quad (4)$$

where **y** and **b** are vectors of phenotypes and fixed effects, respectively, as introduced in Model 1; $\mathbf{u_i}$ is the additive genetic effect with variance of $\mathbf{G_i}\sigma_{u_i}^2$, where $\mathbf{G_i}$ is the genomic relationship matrix constructed from SNPs located on chromosome i, and $\sigma_{u_i}^2$ is the variance explained by SNPs on chromosome i; $\mathbf{u_{all\_without\_i}}$ is the additive genetic effect due to all the SNPs except those on chromosome i; **e** is the residual effect; and **X**, $\mathbf{Z}_1$, and $\mathbf{Z}_2$ are incidence matrices for **b**, $\mathbf{u_i}$ and $\mathbf{u_{all\_without\_i}}$, respectively. The heritability for each chromosome is equal to the ratio of $\sigma_{u_i}^2$ divided by the sum including the variance components from all SNPs on chromosome i plus the variance components from all other SNPs plus the residual variance.

### Gene annotation

The database (version UMD3.1) including gene locations, start positions and end sites for all bovine genes was downloaded from Ensembl [36]. Originally, 24,616 gene ID entries were available in the database. However, only the 17,545 genes on BTA1 to 29 with valid evidences for gene ontology [37, 38] were considered in subsequent analyses. First, SNPs used for GWAS (i.e. the full SNP dataset) were mapped to the genes, by applying the MAGMA software [39], and considering a window

100 kb upstream and downstream for each gene. In the next step, a test statistic for each gene was generated by summing −2log($p$ values) from a set of SNPs within the aforementioned window. This test followed a Chi square distribution [39]. Also, the $p$-value for each of the 17,545 genes was calculated, and further adjusted according to the FDR [32]. Only the genes with a FDR lower than 5% were considered as significantly associated with one of the BW traits. Then, functional classification analyses were conducted for the significant candidate genes, based on information from the PANTHER database [40].

## Results and discussion

### Genetic parameters

Direct pedigree-based heritabilities (i.e. using the **A** -matrix) for BW traits were moderate to high: 0.46 for BW0, 0.37 for BW23, and 0.48 for BW1314 (Table 2). Similar heritabilities were reported in previous quantitative genetic studies for BW [5, 18, 41]. Interestingly, the maternal genetic component had also a moderate contribution, even for BW1314 recorded later in life. Maternal heritabilities of 0.14, 0.11 and 0.13 were found for BW0, BW23, and BW1314, respectively. Although the genomic relationship matrix (**G**) takes the Mendelian sampling term into account, direct genomic heritabilities (Model 2) i.e. 0.33 for BW0, 0.19 for BW23, and 0.22 for BW1314 were lower compared to the pedigree-based heritabilities estimated with the **A**-matrix (Model 1). The lower heritabilities estimated with the **G**-matrix could be due to incomplete LD between SNPs on the 50 K SNP chip and/or very different sample sizes used for the estimations. An explanation for the overestimated pedigree-based heritabilities could be the occurrence of confounding between environmental effects and pedigree relationships [42]. Direct genetic correlations between the three BW traits estimated from genomic relationships (0.51 between BW0 and BW23, 0.33 between BW0 and BW1314, 0.47 between BW23 and BW1314) were slightly higher than those based on pedigree information.

### Population stratification

The k-means clustering approach (using the first 10 PC) created three clusters including 856 cows (cluster 1), 14,305 cows (cluster 2) and 760 cows (cluster 3). Genetic distances between animals based on the two most important PC (the first two PC that contribute to genetic variation) are shown in Fig. 1. Our study included Holstein dairy cattle from only two neighbouring German breeding organizations. When tracing back to the ancestors of the calves and heifers from the three clusters, we found that animals in clusters 1, 2 and 3 were daughters from 2, 890, and 11 sires, respectively. One specific influential sire (Gunnar) in cluster 1 had 855 daughters, whereas another sire (Raik) in cluster 1 had only one daughter in cluster 1 and one daughter in cluster 2 (i.e. the only black dot that overlaps with the red dot in Fig. 1). The 760 calves and heifers in cluster 3 were daughters from 11 different sires. One specific sire (Guarini) had 750 daughters in cluster 3, and the remaining 10 sires only had one daughter each. The maternal grandsire of the nine daughters was Guarini. Sires in cluster 2 originated from various countries, but more than 75% calves and heifers had German and Dutch sires. The remaining 25% females were daughters of sires from 12 other countries. The average number of daughters per sire in cluster 2 was quite small (on average only 16.09). In contrast, the calves and heifers allocated to clusters 1 and 2 were mainly daughters from only two German sires. Consequently, as expected from the pedigree structure, genetic distances between animals within clusters 1 and 2 were short. Hence, the stratification that was observed in the genotyped calves and heifers was mainly due to the size and structure of the half-sib groups. The effect of breeding organization (geographical location) on population

**Table 2 Genetic parameters for body weight recorded at different ages based on pedigree and genomic relationship matrices**

| Relationship matrix | Trait | Heritability | | | Genetic correlation for direct effects | |
|---|---|---|---|---|---|---|
| | | Direct | Maternal | Total | BW23 | BW1314 |
| Pedigree | BW0 | 0.46 (0.01) | 0.14 (0.01) | 0.40 (0.01) | 0.46 (0.03) | 0.39 (0.03) |
| | BW23 | 0.37 (0.01) | 0.11 (0.01) | 0.23 (0.01) | | 0.46 (0.04) |
| | BW1314 | 0.48 (0.02) | 0.13 (0.01) | 0.34 (0.01) | | |
| Genomic | BW0 | 0.33 (0.01) | | | 0.51 (0.05) | 0.33 (0.04) |
| | BW23 | 0.19 (0.02) | | | | 0.47 (0.07) |
| | BW1314 | 0.22 (0.02) | | | | |

Standard errors in parentheses

BW0: body weight recorded at birth; BW23: body weight recorded at 2 to 3 month of age; BW1314: body weight recorded at 13 to 14 months of age

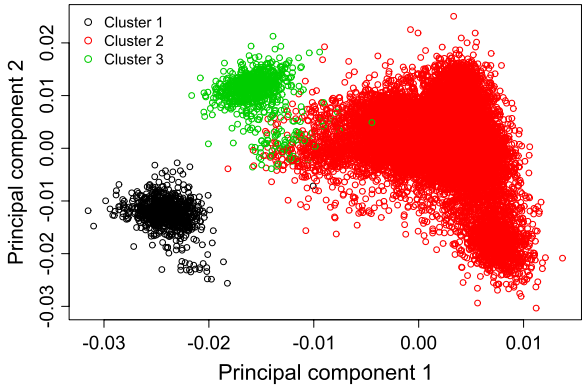


**Fig. 1** Plot of principal components (PC) 1 and 2 for 15,921 genotyped cows

stratification was of minor importance, because genotyped animals in all clusters represented both breeding organisations quite equally.

PC1 and PC2 only explained 1.53 and 1.13% of the total genetic variation, respectively. Consequently, we observed several overlaps between the three clusters, especially for animals allocated to clusters 2 and 3. In other studies, population stratification occurred when various breeds were pooled in the same GWAS [43], or because of obvious differences in breeding and selection strategies [44]. In addition, family structure, especially in large families with many closely related paternal half-sibs, generated false positive SNP effects. In this regard, in a preliminary GWAS without considering any polygenic effects, we detected a large number of more than 2000 significant SNPs (after Bonferroni correction), and the inflation factor was equal to 6.04.

## GWAS for body weights

### *Direct genetic effects*

The number of significant SNPs that contributed to direct genetic effects for the three BW traits (results from Model 3) are listed in Table 3. Evaluation criteria for all similarity matrices are provided for BW0 only. The general trend in terms of number of significant SNPs and inflation factors for BW23 and BW1314 was in agreement with corresponding similarity matrices for BW0. Inflation factors were largest when using LOCO for the construction of the genetic similarity matrix. This was the case for both types of dependent variables, i.e. phenotypes (inflation factor = 2.22) and dDRP (inflation factor = 2.19). The number of significant SNPs and inflation factors decreased slightly when SNPs on BTA1 to BTA11 were partitioned into two segments (LOCO_SEG40). A further decrease in inflation factor was observed when the number of segments (LOCO_SEG441 and LOCO_SEG864) increased, associated with a reduction of significant SNPs. LOCO plus the first 20 PC of the overall **G**-matrix as covariates identified a quite fairly large number of 73 significant SNPs, and contributed to large inflation factors (1.90 for phenotypes and 1.87 for dDRP). The inclusion of 20 PC of chromosome-wide **G**-matrices as fixed regressions in the model (LOCO + CHR_PC20) decreased the number of significant SNPs, and the inflation factor was close to 1. Inflation factors and number of detected significant SNPs were substantially larger for **A** (phenotype: 1213 SNPs according to FDR, $\lambda = 1.92$) compared to **G** (phenotype: 7 SNPs according to FDR, $\lambda = 0.96$). Such obvious differences were not expected, because **G** is the realized relationship matrix and **A** is the expected relationship matrix. Models with **G** and LOCO + CHR_PC produced inflation factors that were equal to 1.0 or slightly lower than 1.0. Generally, a near identical number of significant SNPs was found in the **G** -matrix scenario and LOCO + CHR_PC scenarios. The number of significant SNPs was larger for BW0 than for BW1314 or BW23. Pruning the SNPs according to low LD decreased inflation factors slightly. This was the case for all three BW traits, regardless of whether phenotypes or dDRP were used as dependent variables. For example, when the phenotype of BW0 was the dependent variable, inflation factors decreased from 2.22 (LOCO) to 1.97 (LOCO_prune). The decrease in inflation factor was even smaller for BW23 and BW1314, which indicated that high LD between SNPs was not the main reason for the large number of false positive SNPs in our dataset.

Based on our results, it is imperative to correct for population stratification in the German Holstein population via **G** or **G**-similarities (i.e. the LOCO_CHR_PC-scenarios). GWAS that include multiple breeds and ignore population structure, increased spurious LD, which led to an inflation of false positive signals [43, 45, 46]. Therefore, PC and genetic relationships [15] were included in the GWAS to prevent spurious associations. Yang et al. [34] compared linear mixed models by including or not candidate markers and recommended exclusion of candidate markers from the **G**-matrix because this improved statistical power. However, for the German Holstein population with many closely related animals, LOCO overestimated SNP effects, which indicated that the **G**-matrix from LOCO cannot capture all of the family relatedness. Correlations between the off-diagonal elements from the "full" **G**-matrix and the LOCO **G**-matrix ranged from 0.98 to 1.0, but the LOCO **G**-matrix slightly underestimated the genomic relationships between animals. This underestimation was identified because the regression coefficients were always smaller than 1.0 when regressing relationships from the "full **G**" on relationships from the "LOCO-**G**".

**Table 3 Number of significant SNPs influencing direct genetic effects and inflation factor for body weight recorded at different ages**

| Trait | Dependent variable | Polygenic effect | FDR[a] | Bonferroni[b] | λ |
|---|---|---|---|---|---|
| BW0 | Phen | **A** | 1213 | 64 | 1.92 |
| | | **G** | 7 | 3 | 0.96 |
| | | LOCO | 2268 | 125 | 2.22 |
| | | LOCO_pruned | 394 | 43 | 1.97 |
| | | LOCO_SEG40 | 1811 | 103 | 2.06 |
| | | LOCO_SEG441 | 80 | 17 | 1.29 |
| | | LOCO_SEG864 | 41 | 12 | 1.18 |
| | | LOCO + PC20 | 1132 | 73 | 1.90 |
| | | LOCO + CHR_PC20 | 16 | 7 | 0.93 |
| | dDRP | **A** | 1463 | 104 | 1.96 |
| | | **G** | 15 | 6 | 0.96 |
| | | LOCO | 2212 | 163 | 2.19 |
| | | LOCO_pruned | 414 | 54 | 1.91 |
| | | LOCO_SEG40 | 1802 | 143 | 2.07 |
| | | LOCO_SEG441 | 103 | 23 | 1.28 |
| | | LOCO_SEG864 | 57 | 15 | 1.17 |
| | | LOCO + PC20 | 1180 | 108 | 1.87 |
| | | LOCO + CHR_PC20 | 13 | 8 | 0.90 |
| BW23 | Phen | **A** | 11 | 0 | 1.17 |
| | | **G** | 0 | 0 | 0.99 |
| | | LOCO + CHR_PC20 | 0 | 0 | 0.66 |
| | | LOCO + CHR_PC10 | 0 | 0 | 0.93 |
| | dDRP | **A** | 22 | 5 | 1.33 |
| | | **G** | 3 | 2 | 1.00 |
| | | LOCO + CHR_PC20 | 0 | 0 | 0.70 |
| | | LOCO + CHR_PC10 | 1 | 1 | 0.96 |
| BW1314 | Phen | **A** | 47 | 14 | 1.46 |
| | | **G** | 5 | 3 | 0.97 |
| | | LOCO + CHR_PC20 | 2 | 2 | 0.72 |
| | | LOCO + CHR_PC10 | 7 | 4 | 0.98 |
| | dDRP | **A** | 50 | 17 | 1.45 |
| | | **G** | 7 | 6 | 0.97 |
| | | LOCO + CHR_PC20 | 3 | 3 | 0.72 |
| | | LOCO + CHR_PC10 | 12 | 6 | 0.97 |

FDR: false discovery rate; Bonferroni: Bonferroni correction; λ: inflation factor; BW0: body weight recorded at birth; BW23: body weight recorded at 2 to 3 month of age; BW1314: body weight recorded at 13 to 14 months of age; dDRP: de-regressed proofs for the direct genetic effect; mDRP: de-regressed proofs for the maternal genetic effect; LOCO_pruned: LOCO based on pruned SNPs

[a] Number of significant SNPs according to false discovery rate

[b] Number of significant SNPs according to Bonferroni-correction

Most of the significant SNPs for direct genetic effects on the three BW traits were located on BTA5 (Table 4). Manhattan and Q–Q plots for direct genetic effects for the three BW traits based on different similarity matrices are presented in Additional file 1. For dDRP of BW23, only two SNPs on BTA5 were significant. Both SNPs were detected using the **G**-matrix. SNP *Hapmap60480-ss46526970* was also significant when applying LOCO + CHR_PC. Only two SNPs (*Hapmap60480-ss46526970* and *Hapmap57466-rs29018274*) on BTA5 significantly contributed to the three BW traits. SNP *Hapmap60480-ss46526970* was significant, regardless of the approach applied. However, SNP *Hapmap57466-rs29018274* was significant only when the **G**-matrix was considered. The pleiotropic SNP (*Hapmap60480-ss46526970*) on BTA5, and the significant SNP on BTA18 (*ARS-BFGL-NGS-109285*), also contributed significantly to BW changes in genotyped Holstein dairy cows in the US [47]. On BTA18, SNP *ARS-BFGL-NGS-109285* was significantly associated with body shape, body size, dystocia, longevity, lifetime economic merit [48], and calving difficulty [15]. The four significant SNPs, i.e. *ARS-BFGL-NGS-39379* for BW0 and BW1314, *ARS-BFGL-NGS-5139* for BW0, *ARS-BFGL-NGS-107035* for BW0, and *ARS-BFGL-NGS-109317* for BW0, had a significant impact on BW [49], live weight [50], carcass retail beef yield [43], and hot carcass weight [51] in beef and crossbred beef cattle.

### *Maternal genetic effects*

For maternal genetic effects, only three significant SNPs according to the FDR threshold were identified when using LOCO plus chromosome-wide PC (Table 5). Two SNPs located on BTA4 and one SNP on BTA19 influenced BW0 significantly (Table 6). Regarding maternal genetic effects at later age points for BW23 and BW1314, no significant SNP was detected. The Manhattan plots for maternal genetic effects on BW0 are in Fig. 2. In a study conducted in crossbred beef cattle [51], the significant SNP *ARS-BFGL-NGS-61198* on BTA4 explained 2.67% of the phenotypic variation for lean rate. The significant SNP *Hapmap53086-rs29025958* on BTA19 was identified as a marker for a QTL that controls fat percentage [52]. According to the infinitesimal model for maternal effects on calving performance [15], many genes with small effects influenced the maternal effect on BW. In this regard, the most significant SNP on BTA 19 explained only 0.14% of the mDRP variance for BW0.

Correlations between SNP effects (using DRP and the **G**-matrix in Model 3) for direct genetic and maternal genetic effects were −0.15 for BW0, −0.27 for BW23, and −0.62 for BW1314. Antagonistic correlations between SNP effects for direct genetic and maternal genetic effects for each chromosome were identified for all three BW traits, except for BW0 (0.01) on BTA16 (Fig. 3). In agreement with correlations that take the SNPs on all the chromosomes into account, and in agreement with pedigree-based correlations, antagonistic

**Table 4 Significant SNPs according to Bonferroni correction for direct genetic effects on body weight recorded at different ages**

| SNP | Chr | Position | Ref allele | Effect | BW0 | | BW23 | | BW1314 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Phen | dDRP | Phen | dDRP | Phen | dDRP |
| *INRA-658* | 3 | 29627982 | A | − | | | | | | $X^{c}$ |
| *BTB-01695573* | 4 | 10794285 | C | + | $X^{b}$ | $X^{b}$ | | | | |
| *ARS-BFGL-NGS-3933* | 5 | 105695909 | G | − | | $X^{a}$ | | | | |
| *Hapmap47397-BTA-74925* | 5 | 105744830 | A | − | $X^{ab}$ | $X^{ab}$ | | | | $X^{ac}$ |
| *Hapmap60480-ss46526970* | 5 | 105870613 | C | − | $X^{ab}$ | $X^{ab}$ | | $X^{ac}$ | $X^{ac}$ | $X^{ac}$ |
| *ARS-BFGL-NGS-39379* | 5 | 106269362 | G | − | $X^{ab}$ | $X^{ab}$ | | | $X^{c}$ | $X^{ac}$ |
| *ARS-BFGL-NGS-10732* | 5 | 106780606 | G | − | | | | | $X^{ac}$ | $X^{ac}$ |
| *Hapmap57466-rs29018274* | 5 | 107362671 | A | + | | $X^{a}$ | | $X^{a}$ | | $X^{a}$ |
| *ARS-BFGL-NGS-5139* | 7 | 92474466 | A | + | | $X^{b}$ | | | | |
| *ARS-BFGL-NGS-107035* | 7 | 93007435 | A | + | $X^{b}$ | $X^{ab}$ | | | | |
| *ARS-BFGL-NGS-109285* | 18 | 57589121 | A | + | | $X^{b}$ | | | $X^{ac}$ | $X^{ac}$ |
| *ARS-BFGL-NGS-109317* | 29 | 49906123 | A | + | $X^{b}$ | | | | | |
| *ARS-BFGL-NGS-40378* | 29 | 50296573 | A | + | $X^{b}$ | $X^{b}$ | | | | |

BW0: body weight recorded at birth; BW23: body weight recorded at 2 to 3 month of age; BW1314: body weight recorded at 13 to 14 months of age

The indicated significant SNPs are from runs that consider the following similarity matrices: [a]the genomic relationship matrix **G**, [b]LOCO + CHR_PC20 and [c]LOCO + CHR_PC10

**Table 5 Number of significant SNPs influencing maternal genetic effects and inflation factor for body weight recorded at different ages**

| Trait | Dependent variable | Polygenic effect | FDR[a] | Bonferroni[b] | λ |
|---|---|---|---|---|---|
| BW0 | mDRP | **A** | 26 | 6 | 1.12 |
| | | **G** | 0 | 0 | 0.99 |
| | | LOCO + CHR_PC20 | 0 | 0 | 0.64 |
| | | LOCO + CHR_PC3 | 3 | 0 | 1.00 |
| BW23 | mDRP | **A** | 0 | 0 | 1.04 |
| | | **G** | 0 | 0 | 0.99 |
| | | LOCO + CHR_PC20 | 0 | 0 | 0.59 |
| | | LOCO + CHR_PC3 | 0 | 0 | 0.91 |
| BW1314 | mDRP | **A** | 0 | 0 | 1.08 |
| | | **G** | 0 | 0 | 1.00 |
| | | LOCO + CHR_PC20 | 0 | 0 | 0.56 |
| | | LOCO + CHR_PC3 | 0 | 0 | 0.91 |

FDR: false discovery rate; Bonferroni: Bonferroni correction; λ: inflation factor; BW0: body weight recorded at birth; BW23: body weight recorded at 2 to 3 months of age; BW1314: body weight recorded at 13 to 14 months of age; dDRP: de-regressed proofs for the direct genetic effect; mDRP: de-regressed proofs for the maternal genetic effect

[a] Number of significant SNPs according to false discovery rate

[b] Number of significant SNPs according to Bonferroni-correction

relationships between direct genetic and maternal genetic effects were most obvious for BW1314. If we focus on the functional region on BTA5 (i.e. between 105,445,909 and 107,612,671 bp), the direct-maternal correlations based on the effects of 46 SNPs were equal to −0.04 for BW0, 0.06 for BW23, and −0.87 for BW1314.

### Genomic heritability for each chromosome

Genomic heritabilities for the three BW traits across the 29 bovine autosomes (results from Model 4) are in Fig. 4. For BW0, genomic heritability was highest (0.03) when the SNPs on BTA5 were considered and decreased to 0.001 when those on BTA26 were considered. BTA5 and BTA26 explained 9.92 and 0.31% of the total genomic variance for BW0, respectively. Genomic heritabilities higher than 0.015 were estimated for BTA2, 4, 5, 7, 11, and 25. When comparing chromosomal genomic variances with GWAS results for BW0 (see Additional file 1a and c), the proportion of explained genomic variance increased as the number of significant SNPs per chromosome increased. For BW23, genomic heritabilities were lower than 0.001 for BTA2, 15, and 28, higher than 0.015 for BTA3, 9, 19 and 21, but significant SNPs were detected only on BTA5 (see Additional file 1e). For BW1314, the highest genomic heritability (0.02) was found for BTA7, but significant SNPs were detected on BTA3, 5, 8, 16, and 18 (see Additional file 1g), for which genomic heritabilities were higher than 0.012 for BTA3, 5, 8, and 18 and only 0.007 for BTA16.

Heterogeneous chromosomal contributions were also reported for BW in Korean beef cattle [53]. Consistent with the latter study, we found variations in chromosome-wise BW variances for the same chromosomes at different ages. Hence, such changes in genomic

**Table 6 Significant SNPs according to false discover rate for maternal-genetic effects on body weight recorded at different ages**

| SNP | Chr | Position | Ref. allele | Effect | BW0 mDRP | BW23 mDRP | BW1314 mDRP |
|---|---|---|---|---|---|---|---|
| *ARS-BFGL-NGS-61198* | 4 | 112474006 | A | + | X | | |
| *ARS-BFGL-NGS-107181* | 4 | 114464406 | A | + | X | | |
| *Hapmap53086-rs29025958* | 19 | 37626478 | G | + | X | | |

BW0: body weight recorded at birth; BW23: body weight recorded at 2 to 3 month of age; BW1314: body weight recorded at 13 to 14 months of age

The indicated significant SNPs are from the run that consider the similarity matrix: LOCO + CHR_PC3

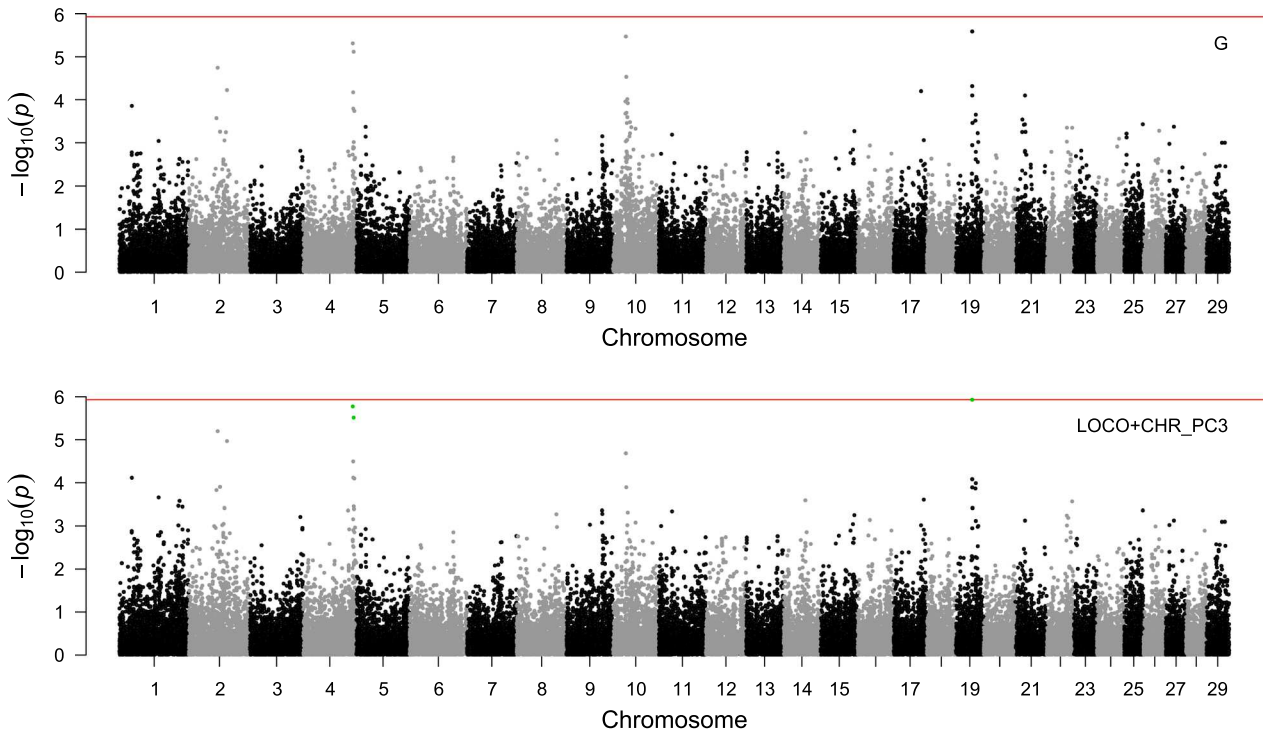


**Fig. 2** Manhattan plot from GWAS for maternal genetic effects on birth weight. **G**: Genomic relationship matrix; LOCO + CHR_PC3: leave-one-chromosome-out plus 3 principal components based on the chromosomal genomic relationship matrix. The red line is the significance threshold line for the Bonferroni correction of 5%, and the green dots represent significant SNPs according to a false discovery rate of 5%

variances indicate that the genetic mechanisms underlying BW differ with age, i.e. that different genes are "switched on or off" during the growth period. We have identified some chromosomes that explain more than 0.015% of the total genomic variance, although no significant SNP was detected (BTA9 for BW23 and BTA7 for BW1314), which indicates polygenic contribution to BW on these chromosomes.

In contrast to [54], we found weak negative covariances between chromosome-wise genomic effects in our data, because the proportions of the sum of chromosome-wise variances to total genomic variances reached 100.81% for BW0, 106.56% for BW23, and 101.41% for BW1314. Linear associations between chromosome length and chromosomal genomic variances were weak for BW0 and BW1314, with $R^2$ values of 0.20 and 0.21, respectively, and null for BW23 ($R^2 = 0.02$). Weak associations between chromosome length and chromosomal genomic variances indicate that the QTL for BW are not evenly distributed across the genome [54].

## Gene annotation

### *Direct genetic effect*

The identified potential candidate genes that significantly influence direct genetic effects on BW are in Additional file 2. These candidate genes are located on 12 chromosomes: BTA3, 4, 5, 7, 8, 11, 13, 18, 19, 23, 25 and 29,

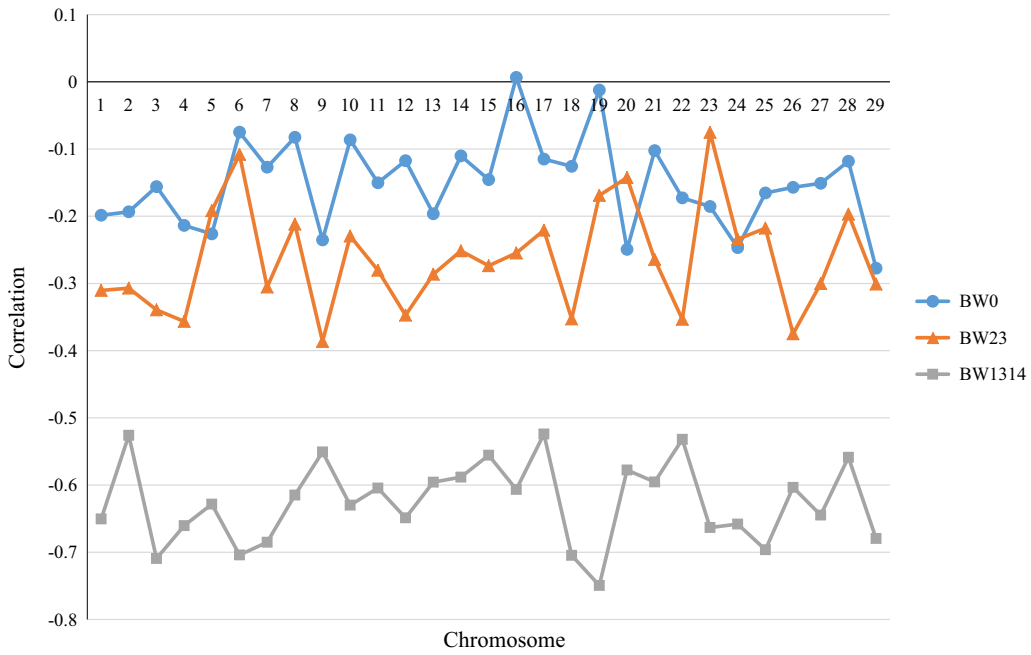


**Fig. 3** Correlations between direct genetic and maternal genetic marker effects for body weight recorded at birth (BW0), at 2 to 3 months of age (BW23) and at 13 to 14 months of age (BW1314)

which BTA5 and BTA18 carrying more than ten. Overall, for the three BW traits, 76 potential candidate genes had adjusted *p* values lower than 0.05 (according to FDR), with 51 significant genes for BW0, 12 for BW23, and 38 for BW1314; these figures reflect the smaller number of significant SNPs detected in the GWAS for BW at later ages. Six genes contributed significantly to the three BW traits and 12 more contributed to both BW0 and BW1314, but only one more gene, i.e. *fast skeletal muscle troponin T* (*TNNT3*) had a significant effect on both BW0 and BW23. Low to moderate genetic correlations between BW traits at different age points, but with some overlapping between significant genes, could indicate pleiotropic effects of the candidate genes.

Some of the potential candidate genes on BTA18 for BW traits are known to be involved in calving performance and conformation traits. For example, Abo-Ismail et al. [16] reported that *cytosolic thiouridylase subunit 1* (*CTU1*) and *ENSBTAG00000037537* are highly associated with body conformation traits and *kallikrein related peptidase 4* (*KLK4*), *CTU1* and *ENSBTAG00000004608* contributes to calving ease. Purfield et al. [15] showed that *CTU1* and *ENSBTAG00000037537* contain one and two significant missense variants, respectively, that are associated with calving difficulty in a mixed bull population including Holstein–Friesian, Charolais and Limousin. Since the above-mentioned six genes also influence birth weight, the calving difficulties in these breeds are mainly due to increased BW of the newborn [55].

Our analyses revealed that the identified potential candidate genes were involved in 12 biological processes (Fig. 5): cellular processes (30 genes), metabolic mechanisms (14 genes), biological regulations and responses to stimuli (10 genes), growth (one gene), and body developmental processes (four genes). The latter four genes were *fibroblast growth factors 6* (*FGF6*) and *fibroblast growth factors 23* (*FGF23*), *fast skeletal muscle troponin T* (*TNNT3*), and o*steomodulin* (*OMD*). *FGF6* and *FGF23* belong to the fibroblast growth factor family, which plays an important role in a variety of biological processes, including angiogenesis, morphogenesis, tissue regeneration, and oncogenesis [40]. Another significant gene, i.e. *cathepsin D* (*CTSD*) is involved in the activation and degradation of polypeptide hormones and growth factors [56]. *TNNT3* produces troponin T protein in the mammalian fast skeletal muscle, with causal effects on $Ca^{2+}$ muscle contractions [57]. *OMD* regulates the diameter and shape of collagen fibrils, which suggest an effect on bone formation [58].

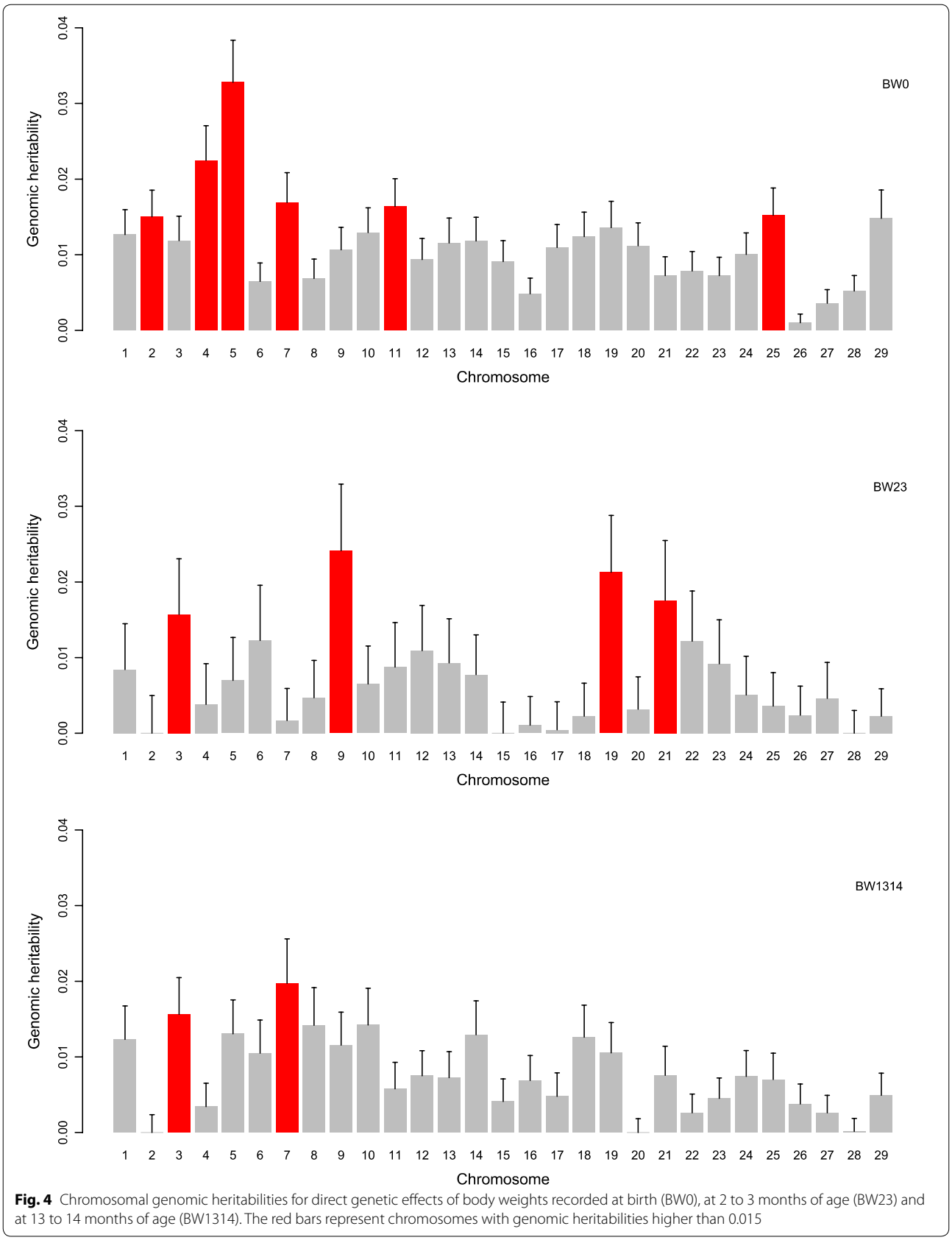


**Fig. 4** Chromosomal genomic heritabilities for direct genetic effects of body weights recorded at birth (BW0), at 2 to 3 months of age (BW23) and at 13 to 14 months of age (BW1314). The red bars represent chromosomes with genomic heritabilities higher than 0.015

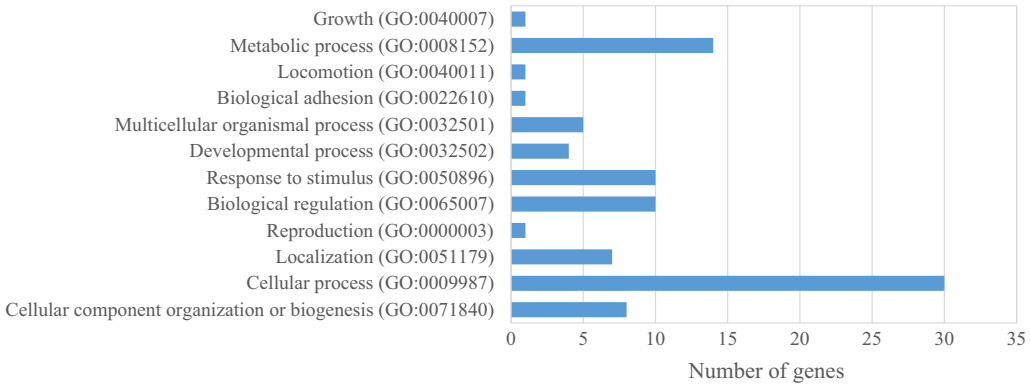


**Fig. 5** Biological processes for direct genetic effects on body weight at different ages

### *Maternal genetic effect*

Four potential candidate genes on BTA19, i.e. *solute carrier family 35 member B1* (SLC35B1), *speckle-type POZ protein* (*SPOP*), *neurexophilin 3* (*NXPH3*), and *nerve growth factor receptor* (*NGFR*), were significantly associated with birth weight (see Additional file 3), although only one significant SNP was detected on BTA19. The biological functions of *SLC35B1* and *NXPH3* remain unknown. *SPOP* is an important regulator of luminal epithelial cell proliferation [59] and is associated with various cancers. *NGFR* affects cell growth and survival [60]. None of these four genes overlapped with the candidate genes identified for direct genetic effects.

## Conclusions

Ignoring the population structure of Holstein–Friesian in the GWAS increased the number of false positive SNPs. Population structure was corrected properly when using **G** and LOCO plus chromosome-wide PC in the statistical models for the GWAS. The number of significant SNPs increased when DRP instead of phenotypes were used as dependent variables. Two SNPs on BTA5 influenced direct genetic effects significantly for BW at the three ages measured. Chromosomes with a larger number of significant SNPs had higher direct chromosomal heritabilities. Gene annotation analysis identified 76 potential candidate genes that are involved in 12 biological processes, which indicates that weight development is a very complex biological process. Regarding birth weight, only a limited number of significant SNPs and candidate genes were identified for the maternal genetic effects, which suggests an infinitesimal model for these effects. Antagonistic associations between direct genetic and maternal genetic effects were observed both when SNPs on all bovine chromosomes or on single chromosomes were considered, and for potential functional regions on BTA5.

## Additional files

**Additional file 1.** Manhattan plots and Q–Q plots from GWAS for birth weight phenotypes (a and b), for birth weight de-regressed proofs (c and d), for BW23 phenotypes and de-regressed proofs (e and f), and for BW1314 phenotypes and de-regressed proofs (g and h). Description: **A** is the pedigree-based relationship matrix; **G** is the genomic relationship matrix; LOCO is leave-one-chromosome-out (LOCO); LOCO_SEG864 is leave one segment out; LOCO + PC20 is LOCO plus 20 principal components; LOCO + CHR_PC20 is LOCO plus 20 principal components based on the chromosomal genomic relationship matrix. The red line is the significance threshold line for the Bonferroni correction of 5%, and the green dots represent significant SNPs according to ae false discovery rate of 5%.

**Additional file 2.** Potential candidate genes for direct genetic effects on body weight recorded at birth (BW0), at 2 to 3 months of age (BW23) and at 13 to 14 months of age (BW1314).

**Additional file 3.** Potential candidate genes for maternal genetic effects on body weights recorded at birth (BW0), at 2 to 3 months of age (BW23) and at 13 to 14 months of age (BW1314).

#### Authors' contributions

TY conducted the genetic statistical analyses and wrote the first draft of the manuscript. SK conceived the ideas for this study and assisted in writing the manuscript. All authors read and approved the final manuscript.

#### Competing interests

The authors declare that they have no competing interests.

#### Consent for publication

Not applicable.

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## 2.6. Original research paper 6

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# Genomic predictions of growth curves in Holstein dairy cattle based on parameter estimates from nonlinear models combined with different kernel functions

**T. Yin and S. König***
Institute of Animal Breeding and Genetics, Justus-Liebig-University Gießen, 35390 Gießen, Germany

## ABSTRACT

Availability of longitudinal body weight (BW) records allows the application of nonlinear models (NLINM) to predict phenotypic and genomic growth curves in dairy cattle. In this regard, we considered a data set including 31,722 BW records from 4,952 female Holstein cattle, during the period from birth (mo 0) to approximately age at first calving (mo 24). Parameters of the growth curves were estimated using 3 NLINM: the logistic (LOG), the Gompertz (GOM), and the Richards (RICH) functions. Residuals for the growth curve parameters from the NLINM applications were used as pseudo-phenotypes in the ongoing genomic analyses with different similarity matrices, including 2 genomic relationship matrices (**G1** and **G2**), a combined pedigree and genomic relationship matrix (**H**), and 3 kernel matrices. The kernels were a weighted "alike by state" kernel function (**K1**), an exponential dissimilarity kernel (**K2**), and a Gaussian kernel (**K3**). On the basis of **G1** and **G2** matrices, genomic heritabilities for the growth curve parameters birth weight ($W_0$), mature weight ($W_m$), and growth rate ($k$), and the shape parameter ($m$; only available from RICH) were moderate to large, in the range from 0.29 ($m$ from RICH) to 0.46 ($k$ from RICH). Fitting the similarity matrices based on kernel functions contributed to an increase of the ratio of the variance explained by the similarity matrix in relation to the total variance (compared with the heritability when modeling **G1** or **G2**). Genetic correlations between $W_0$, $W_m$, and $k$ were always positive (>0.30), especially for the same growth curve parameters estimated from different NLINM (>0.90). The shape parameter $m$ from RICH was negatively correlated with other growth curve parameters, from −0.29 to −0.95. In a next step, estimated genomic breeding values for growth curve parameters were input data for the respective NLINM, aiming to construct genomic growth curves. Prediction accuracies were correlations between genomic growth curves and genomic breeding values from random regression models for sires and female cattle. Considering all genotyped female cattle with pseudo-phenotypes, prediction accuracies were larger from RICH than from LOG and GOM. However, differences in prediction accuracies from the NLINM × similarity matrix combinations were quite small. Accordingly, in 5-fold cross-validations using heifer groups with masked phenotypes, very similar prediction accuracies across modeling approaches were identified. Especially for specific age months, genomic growth curve predictions were more accurate for sires than for female cattle, indicating that the relationships between animals in training and validation sets are more important than the selection of specific NLINM × similarity matrix combinations.

**Key words:** longitudinal body weight, nonlinear models, growth curve parameters, genomic predictions

## INTRODUCTION

Body weight is a trait of increasing importance in dairy cattle breeding programs. Classically, BW was used to determine feed requirements for maintenance and production demands (Visscher et al., 1994). Recently, Pryce et al. (2015) suggested controlling of maintenance costs and improvements for feed efficiency through the optimal combination of BW and production traits in selection index applications. Similarly, Connor (2015) mentioned the possibilities of predicting maintenance costs when simultaneously considering residual feed intake and mature live weight. Consequently, due to the strong influence of BW on feed efficiency traits, dairy cattle breeders have requested inclusion of BW in overall breeding goals, especially in pasture-based production systems (Pryce and Harris, 2006). Furthermore, BW is genetically favorably correlated with production and fertility traits (Berry et al., 2003; Yin and König,

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*Corresponding author: sven.koenig@agrar.uni-giessen.de

2018), indicating indirect selection response in a broad pattern of trait categories when incorporating BW in selection indexes.

Automatic weighing systems, as installed in modern dairy cattle farming systems, allow generation of a dense longitudinal BW data structure along the growth trajectory. For genetic analyses of repeated BW measurements, Yin and König (2018) applied linear repeatability and linear random regression models (**RRM**) with continuous time-dependent covariates. For the prediction of BW at specific time points and the modeling of individual growth curves, specific nonlinear functions have also been considered. For instance, Koskan and Ozkaya (2014) modeled growth curves for female Holstein cattle by applying the Brody (Fitzhugh, 1976), Gompertz (France et al., 1996), Logistic (France et al., 1996), Richards (France et al., 1996), and von Bertalanffy functions (von Bertalanffy, 1957). A comprehensive evaluation of the 5 nonlinear models (**NLINM**) was carried out via meta-analysis (Teleken et al., 2017), considering growth data from 14 different publications. Teleken et al. (2017) indicated that estimated parameters from NLINM reflect biological characteristics such as mature weight and maturing rate. However, irrespective of their flexibility, the complexity of NLINM hampered their applications for phenotypic and genetic BW predictions at specific points in time.

Availability of high-density SNP marker panels and sequencing data from genotyped cows allows consideration of alternative approaches regarding genetic-statistical modeling and genomic predictions via NLINM. An obvious problem for estimations of SNP effects with linear regression models, especially for novel functional traits, is the substantially smaller number of genotyped cows compared with the number of SNP. Alternatively, in nonparametric regressions (Gianola and van Kaam, 2008), all or a subset of the markers (**X**) can be used to build the variance-covariance structure between individuals through the definition of a nonparametric function g(**X**). The function g(**X**) can be specified by reproducing kernel Hilbert space regression (Gianola and van Kaam, 2008). According to the Representer theorem (Kimeldorf and Wahba, 1971), the solution of $g(\mathbf{X}) = \mathbf{K}\boldsymbol{\alpha}$, where **K** is a n-by-n kernel matrix (n = number of genotyped animals) and $\boldsymbol{\alpha} = (\alpha_1, \ldots, \alpha_n)$ is a vector of unknown parameters. Element $(i, j)$ in **K** is $K(x_i, x_j)$, which is calculated by the kernel function, based on the genotypes of animal $i$ and animal $j$. Hence, the input marker information is linked to the traits only through the kernel matrix **K** as produced from the kernel function. Schaid (2010a,b) reviewed various kernel functions, including the weight linear kernel (Wu et al., 2011), the polynomial kernel (Zien et al., 2000), and the Gaussian kernel (Mallick et al., 2005). The general characteristics of all kernels are identified similarities or dissimilarities between individuals, based on, for instance, genotypes, amino acids, or gene expressions. Additionally, when a pedigree-based (**A**) matrix or a marker-based (**G**) relationship with almost 50,000 SNP is chosen as a kernel, a classical additive infinitesimal model in quantitative genetics is depicted (de los Campos et al., 2010a). However, in contrast with **A** and **G** matrices, the variance-covariance structure built on specific kernel functions, such as the polynomial kernel or the Hadamard product of 2 kernel matrices, captures additive genetic as well as nonlinear effects, such as SNP marker interactions (Wang et al., 2015).

To the best of our knowledge, no studies are available that combine parameters from NLINM with genomic information in specific kernel functions for the prediction of growth curves. Therefore, the objectives of the present study were as follows: (i) to use longitudinal BW data from Holstein heifers for the modeling of phenotypic growth curves, considering 3 nonlinear growth curve functions (logistic, Gompertz, and Richards models); (ii) to estimate genomic breeding values (**GBV**) of the growth curve parameters from the 3 nonlinear functions based on 2 **G** matrices and 3 similarity kernels (weighted "alike by state" kernel, exponential dissimilarity kernel and Gaussian kernel); and (iii) to assess the prediction accuracies of genomic growth curves.

## MATERIALS AND METHODS

### Phenotype Data

Data editing for BW followed the protocol by Yin and König (2018). In this regard, we excluded levels of herd-year-month effects with fewer than 10 BW records and BW outliers based on studentized residuals and corresponding Bonferroni $P$-values $< 0.05$ or $> 0.95$. Body weights of 4,952 female Holstein cattle were recorded repeatedly during a period from birth (mo 0) to 24 mo (approximately age at first calving). The total number of BW records was 31,722, implying on average 6.41 records per animal (range: 4 to 19 repeated measurements). The cattle were kept in 10 large-scale herds, located in the German federal states of Mecklenburg-West Pomerania and Berlin-Brandenburg, and they were born between 2005 and 2014. The mean, minimum, maximum, and standard deviation for BW were 252.75 kg, 20 kg, 742 kg, and 168.63 kg, respectively. Pedigree after pruning included 20,176 animals, born between 1955 and 2014. On average, every dam had 1.22 phenotyped daughters within a range of 1 to 4. Number of daughter BW records

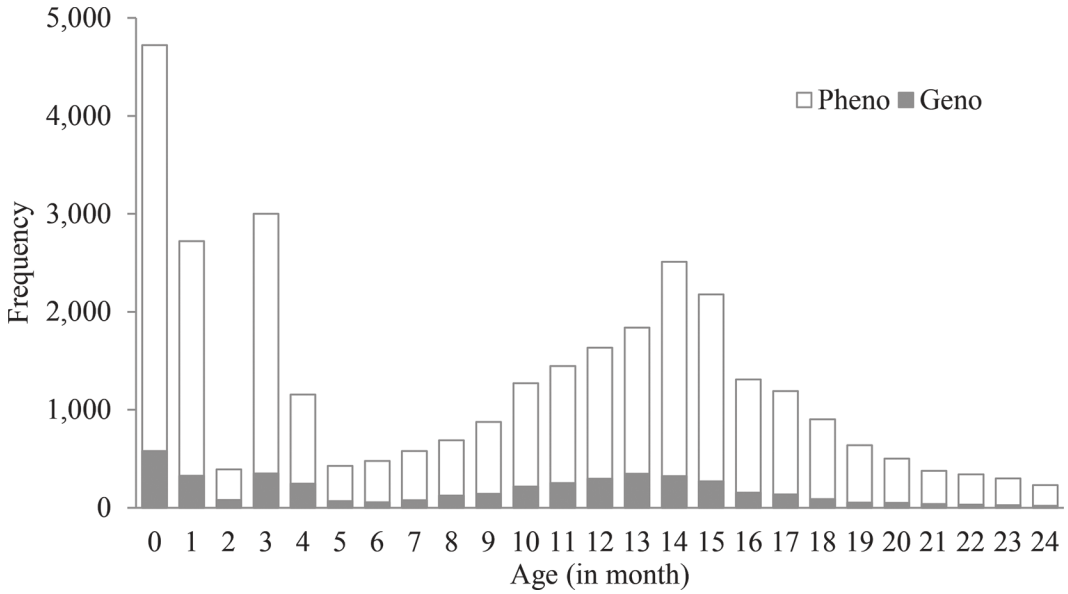


**Figure 1.** Number of observations per growth month for all cattle with phenotype BW records (Pheno, 4,952 cattle) and for genotyped cattle with BW records (Geno, 620 cattle).

per dam ranged from 4 to 45, and the average was 7.82. The 4,952 Holstein heifers were daughters from 780 sires, 1 to 173 phenotyped daughter per sire. The number of daughter records per sire ranged from 4 to 1,073 (average: 40.67 daughter records per sire). The distribution of BW records by age is given in Figure 1 for the heifers with phenotypes and for the heifers with phenotypes and genotypes.

### Genotype Data

Among the female cattle with phenotypic data, 620 were genotyped using the Illumina Bovine 50K SNP BeadChip V2 (161 animals), or the Illumina Bovine Eurogenomics 10K low-density chip (459 animals; Illumina, San Diego, CA). Additionally, 1,101 sires of 4,952 calves and heifers were also genotyped with the Illumina Bovine 50K SNP BeadChip V2 (180 sires) or the Illumina Bovine Eurogenomics 10K low-density chip (921 sires). The 10K SNP genotypes were imputed to the 50K SNP panel by project partner vit (Verden, Germany), as done for national routine genetic evaluations (Segelke et al., 2012). Genotype quality controls were performed using PLINK software, version 1.9 (Chang et al., 2015; Purcell and Chang, 2019). Filtering criteria for markers were as follows: consideration only of SNP located on *Bos taurus* autosomes; minor allele frequency larger than 0.01; minimum call rate of 0.95; and no significant deviation from Hardy-Weinberg equilibrium ($P > 0.0001$). Finally, 1,721 genotyped animals with 44,314 SNP were included in genomic analyses.

### Nonlinear Growth Functions

Three frequently used nonlinear growth functions (Table 1), including two 3-parameter functions and one 4-parameter function, were employed to model the growth curves of the phenotyped female cattle. The 3-parameter functions were the logistic (**LOG**) and the Gompertz (**GOM**) models, both with the characteristics of a sigmoidal shape and a fixed inflection point. The fixed inflection points of the 2 models are located at about 50% (LOG) and 37% (GOM) of the mature weight (Teleken et al., 2017). The curve parameters with biological meanings of LOG and GOM models are the birth weight ($\boldsymbol{W_0}$), the mature body weight ($\boldsymbol{W_m}$), and a constant growth rate ($\boldsymbol{k}$) reflecting an average velocity from birth to mature weight. The 4-parameter Richards (**RICH**) function embraces all the parameters from LOG and GOM. The auxiliary parameter is a shape parameter ($\boldsymbol{m}$), indicating associations between proportions of mature weight with the inflection point (Gbangboche et al., 2008).

The "saemix" function from the R package "saemix" (Comets et al., 2017), with an implemented stochastic approximation expectation maximization algorithm, was used to estimate parameters of the 3 NLINM. The equation for the models with homogeneous residuals was as follows:

$$y_{ij} = f\left(t_{ij}, \Psi_i\right) + e_{ij}, \quad [1a]$$

and when assuming an exponential error, the model was as follows:

$$\log\left(y_{ij}\right) = \log\left[f\left(t_{ij}, \Psi_i\right)\right] + e_{ij}, \quad [1b]$$

where $y_{ij}$ = the $j$th BW observation for animal $i$; $f(t_{ij}, \Psi_i)$ = one of the nonlinear functions; $t_{ij}$ = age of animal $i$ when the $j$th observation was recorded; $\Psi_i$ = individual parameters for the nonlinear functions with $\Psi_i = \Psi_{\text{pop}} + \sum_{l=1}^{9} h_{il}\beta_l + \eta_i$, where $\Psi_{\text{pop}}$ = one of the population growth curve parameters (overall mean), $h_{il}$ = dummy variables for the herd effect $l$ (only the herd effect was included, due to convergence problems), $\beta_l$ = estimates for a single herd $l$ compared with the reference herd (herd no. 10), $\eta_i$ = random effects for each animal, $N(\mathbf{0},\mathbf{\Omega})$; $e_{ij}$ = random residual effects with $e_{ij} \sim N\left(0, \sigma_e^2\right)$. The subscripts varied from 1 to 4,952 (number of animals) for $i$ and from 4 or 19 (no. of repeated measurements per animal) for $j$. $\boldsymbol{\Psi}_{\mathbf{pop}}$ was a vector with $d$ elements, and $\mathbf{\Omega}$ was a $d \times d$ variance-covariance matrix. For the 3-paramater functions (LOG and GOM), $d$ was 3, and for RICH, $d$ was 4. Model evaluation criteria were the −2 times maximum log-likelihood (−2LL), the Akaike information criterion and the Bayesian information criterion, which are all output from the applied "saemix" function. Low values for −2LL, Akaike information criterion, and Bayesian information criterion indicate model superiority.

In the first step, population parameters ($\boldsymbol{\Psi}_{\mathbf{pop}}$), variance-covariance matrices ($\mathbf{\Omega}$), residual variances $\left(\sigma_e^2\right)$, and coefficients for the herd effect were estimated. Afterward, the individual growth curve parameters ($\Psi_i$) were calculated based on 100 samples from the conditional distribution $\mathrm{p}\left(\Psi_i \mid y_i; \widehat{\Psi_{\text{pop}}}; \hat{\Omega}; \widehat{\sigma_e^2}\right)$. Due to the fact that $\Psi_i$ as estimated from the R package "saemix" comprised the herd effect again, the conditional means of each individual were further adjusted for herd and birth year. Thus, we applied a weighted least squares model, which incorporated the respective conditional variances. The statistic model in scalar notation was

$$y_{ijk} = h_i + b_j + e_{ijk}, \quad [2]$$

where $y_{ijk}$ = conditional means of individual parameters for animal $k$ within herd $i$ and birth year $j$; $h_i$ = herd $i$; $b_j$ = birth year $j$; and $e_{ijk}$ = random residual effects with $e_{ijk} \sim N\left(0, W_k\sigma_e^2\right)$, where $W_k$ = reciprocals of conditional variance for animal $k$. Residuals for the growth curve parameters from the weighted least squares analyses were used as pseudo-phenotypes in the ongoing genomic analyses.

## Genetic Parameter Estimations

The residuals of 10 curve parameters (3 for LOG, 3 for GOM, and 4 for RICH) from 620 genotyped female cattle were used as pseudo-phenotypes in animal models to estimate genomic curve coefficients for all animals. We applied the REML algorithm as implemented in the DMU v. 6 software package (Madsen and Jensen, 2013). The statistical model was

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{g} + \mathbf{e}, \quad [3]$$

where $\mathbf{y}$ = a vector of pseudo-phenotypes; $\mu$ = the overall mean; $\mathbf{g}$ = a vector of random polygenic effects

**Table 1.** Applied growth functions for the modeling of growth curves

| Model | Equation[1] | Reference |
|---|---|---|
| Logistic | $W\left(t\right) = \frac{W_m}{1 + \left[\left(\frac{W_m}{W_0}\right) - 1\right]e^{-kt}}$ | France et al., 1996 |
| Gompertz | $W\left(t\right) = W_m e^{\ln\left(\frac{W_0}{W_m}\right)e^{-kt}}$ | Teleken et al., 2017 |
| Richards | $W\left(t\right) = \frac{W_m W_0}{\left[W_0^m + \left(W_m^m - W_0^m\right)e^{-kt}\right]^{\frac{1}{m}}}$ | France et al., 1996 |

[1] $W(t)$ = BW at age $t$; $W_0$ = birth weight; $W_m$ = mature BW; $k$ = constant growth rate; $m$ = shape parameter; $t$ = age in months, and $e$ = a mathematical constant.

with a variance-covariance structure of $\mathbf{g} \sim N\left(0, \mathbf{K}\sigma_g^2\right)$, where $\mathbf{K}$ = the similarity matrix between individuals, $\sigma_g^2$ = the polygenic variance, and $\mathbf{e}$ = a vector of random residual effects with $\mathbf{e} \sim N\left(0, \mathbf{I}\sigma_e^2\right)$.

Using the genomic marker data, 5 similarity matrices were constructed. First, we created $\mathbf{K}$ according to the genomic relationship matrix as introduced by VanRaden (2008). Therefore, $\mathbf{G}_1 = \frac{\mathbf{ZZ}'}{\sum 2p_k\left(1-p_k\right)}$, where $\mathbf{Z} = \mathbf{M} - 2\mathbf{P}$ with $\mathbf{M}$ = genotypes (coded as 0, 1, or 2) of the animals; $\mathbf{P}$ = a matrix with allele frequency for marker $k$ in column $k$; $p_k$ = allele frequency for marker $k$ in the genotyped population ($k$ varied from 1 to 44,314). The second similarity matrix followed the same rules, but the centralized $\mathbf{Z}$ matrix was standardized with $\sqrt{2p_k\left(1-p_k\right)}$, implying $\mathbf{G}_2 = \frac{\mathbf{ZDZ}'}{p}$, where $p$ = the number of markers after filtering (44,314) and $\mathbf{D}$ = a $p$-by-$p$ diagonal matrix with $\frac{1}{2p_k\left(1-p_k\right)}$ on the diagonal (Amin et al., 2007). To avoid singularities of $\mathbf{G1}$ and $\mathbf{G2}$, a small value of 0.02 was added to the diagonal of both matrices.

In addition to the 2 genomic relationship matrices $\mathbf{G_1}$ and $\mathbf{G_2}$, 3 kernel matrices were constructed to measure the similarities in terms of genomic marker conformity among all individuals. The weighted "alike by state" kernel function, as introduced by Wu et al. (2011), was defined as the first kernel ($\mathbf{K1}$). The respective formula is as follows: $\mathbf{K1}(\mathrm{i},\mathrm{j}) = \sum_{k=1}^{p} w_k\left(2 - \left|x_{ik} - x_{jk}\right|\right)$, where $p$ was the number of markers; $x_{ik}$ was the genotype of the $k$th marker for animal $i$; and $w_k = \frac{1}{\sqrt{p_k\left(1-p_k\right)}}$. Hence, the allele difference of every SNP was weighted considering the respective allele frequency (Wang et al., 2015). For the exponential kernel $\mathbf{K2}$ based on a dissimilarity score ($\mathbf{S}$), we used the following algorithm from González-Recio et al. (2008): (1) calculation of frequencies for marker $k$ and genotype $s$ ($f_{ks}$), implying 1 of the 3 frequencies for every marker corresponding to genotypes 0, 1, and 2 when genotypes for animal $i$ and $j$ at the $k$th marker are identical; (2) calculation of the dissimilarity score S between animal $i$ and $j$ for the $k$th marker on chromosome 1

$$\begin{cases} T_k = T_{k-1}f_{ks} \text{ and } S_k = S_{k-1}; \text{if } x_{ik} = x_{jk} \\ S_k = S_{k-1} + T_{k-1} \text{ and } T_k = 1; \text{if } x_{ik} \neq x_{jk} \end{cases},$$

where $T_k$ = temporary dissimilarity score for the $k$th marker, $x_{ik}$ = genotype for animal $i$ and marker $k$, $T_0 = 1$, and $S_0 = 0$; the loop ran from the first to the last SNP on chromosome 1, resulting in $\mathrm{S_{chr1}}$; (3) calculation of the dissimilarity score S as described in step 2 for all the other chromosomes, one by one; and (4) calculation of the overall $\mathrm{S} = (\mathrm{S_{chr1}} + \ldots + \mathrm{S_{chr29}})/\mathrm{p}$. The exponential kernel was calculated as $\mathbf{K2} = \exp(-\mathrm{S})$.

Another exponential kernel, that is, the Gaussian kernel ($\mathbf{K3}$), was formed as

$$\mathbf{K3}(i,j) = \exp\left(-\frac{\left\|x_i - x_j\right\|^2}{\delta}\right) = \exp\left(-\frac{\left(x_i - x_j\right)'\left(x_i - x_j\right)}{\delta}\right),$$

where $\delta$ was a bandwidth parameter. The optimal bandwidth parameter was chosen among a grid of values ranging from $0.1 \times \max\left\{\left\|x_i - x_j\right\|^2\right\}$ to $1.0 \times \max\left\{\left\|x_i - x_j\right\|^2\right\}$ with an interval of 0.1. Evaluation of the bandwidth parameters was carried out using the R package "rrBLUP" (Endelman, 2011) with respect to the log-likelihood of the model when fitting different $\delta$ to scale the kernel matrix. Generally, for all the growth parameters, the log-likelihood was maximized when $\delta$ was $0.8 \times \max\left\{\left\|x_i - x_j\right\|^2\right\}$. Therefore, the optimal bandwidth for $\mathbf{K3}$ in this study was always set to $0.8 \times \max\left\{\left\|x_i - x_j\right\|^2\right\}$.

Additionally, genomic growth curve parameters for all female cattle (4,952) were estimated via genomic animal models, applying REML as implemented in the software package DMU v. 6 (Madsen and Jensen, 2013):

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{Zu} + \mathbf{e}, \quad [4]$$

where $\mathbf{y}$, $\mu$, and $\mathbf{e}$ were the same as defined in model 3; $\mathbf{u}$ = a vector of estimated GBV with $\mathbf{u} \sim N\left(\mathbf{0}, \mathbf{H}\sigma_u^2\right)$; $\sigma_u^2$ = the genetic variance; and $\mathbf{Z}$ = incidence matrix for $\mathbf{u}$. The combined $\mathbf{H}$ matrix was computed by blending $\mathbf{A}$ and the weighted genomic relationship matrix ($\mathbf{G_w}$; Legarra et al., 2009). $\mathbf{G_w}$ was calculated as follows: $\mathbf{G_w} = \left(0.95 \times \mathbf{G_1} + 0.05 \times \mathbf{A_{22}}\right)$, where $\mathbf{A_{22}}$ is the submatrix of the pedigree-based relationship matrix for genotyped animals. Heritabilities of the growth curve parameters were estimated from univariate models. Genetic and phenotypic correlations among all growth

**Table 2.** Descriptions of the models as applied in the present study[1]

| Model | Model no. | Dependent variable | Similarity matrix | No. of animals | No. of observations |
|---|---|---|---|---|---|
| $y_{ij} = f\left(t_{ij}, \Psi_i\right) + e_{ij}$ | [1a] | Longitudinal BW | — | 4,952 | 31,722 |
| $\log\left(y_{ij}\right) = \log\left[f\left(t_{ij}, \Psi_i\right)\right] + e_{ij}$ | [1b] | | | | |
| $y_{ijk} = h_i + by_j + e_{ijk}$ | [2] | Individual growth curve parameters from model 1b | — | 4,952 | 4,952 |
| $\mathbf{y} = \mathbf{1}\mu + \mathbf{g} + \mathbf{e}$ | [3] | Residuals from model 2 | **G1**, **G2**, **K1**, **K2**, and **K3** | 620 | 620 |
| $\mathbf{y} = \mathbf{1}\mu + \mathbf{g} + \mathbf{e}$ | [4] | Residuals from model 2 | **H** | 4,952 | 4,952 |
| $\mathbf{y} = \mathbf{Xb} + \mathbf{Qd} + \mathbf{Wm} + \mathbf{Zp} + \mathbf{Sp}_m + \boldsymbol{e}$ | [5] | Longitudinal BW | **H** | 4,952 | 31,722 |

[1]**G1** = genomic relationship matrix according to VanRaden, 2008; **G2** = genomic relationship matrix according to Amin et al., 2007; **K1** = kernel matrix according to Wu et al., 2011; **K2** = kernel matrix according to González-Recio et al., 2008; **K3** = Gaussian kernel; **H** = combined matrix blending **A** and the weighted genomic relationship matrix according to Legarra et al., 2009; $\sigma_g^2$ = variance explained by the similarity matrix.

curve parameters were estimated with model 3, implying series of bivariate runs for all trait combinations. Standard errors for phenotypic and genetic correlations were calculated based on the estimated (co)variance component matrix, using the "deltamethod" function from the R package "msm" (Jackson, 2019).

## Genomic Random Regression Model

Data basis was 31,722 BW records from 4,952 heifers. A Bayesian approach, as implemented in the DMU v. 6 package (Madsen and Jensen, 2013), for genomic RRM was applied to estimate genomic breeding values (**GBV-RRM**) for BW at specific age months. The RRM was defined according to Yin and König (2018):

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Qd} + \mathbf{Wm} + \mathbf{Zp} + \mathbf{Sp_m} + \mathbf{e}, \quad [5]$$

where $\mathbf{y}$ = vector of observations for longitudinal BW; $\mathbf{b}$ = vector of fixed effects including herd-year-month, and regressions on age in month using cubic Legendre polynomials nested within birth year; $\mathbf{d}$ = vector of direct additive genetic effects for random regression coefficients, which were modeled with Legendre polynomials of order 3; $\mathbf{p}$ = vector of permanent environmental effects for random regression coefficients, which were modeled with Legendre polynomials of order 3; $\mathbf{m}$ = vector of maternal genetic effects; $\mathbf{p_m}$ = vector of maternal permanent environmental effects; $\mathbf{e}$ = vector of random residual effects; $\mathbf{S}$ = incidence matrices for $\mathbf{b}$ and $\mathbf{p_m}$, respectively; and $\mathbf{X}$, $\mathbf{Q}$, $\mathbf{W}$, and $\mathbf{Z}$ = coefficient matrices for $\mathbf{d}$, $\mathbf{m}$, and $\mathbf{p}$, respectively. Heterogeneous residual variances were assumed across the age scale for the following time intervals: 0, 1 to 4, 5 to 8, 9 to 12, 13 to 16, 17 to 20, and 21 to 24 mo. Again, the genetic relationships were constructed using the **H** matrix (Legarra et al., 2009) as explained above.

In the Bayesian framework, we considered a chain length of 300,000 iterations, a burn-in period of 60,000 rounds, and a thinning interval of 10. Effective sample sizes of the estimates ranged from 23.1 for the maternal permanent environmental effect to 21,650.6 for the residual component in mo 5 to 8. Estimated random regression coefficients for direct genetic and permanent environmental effects were averaged, considering the 24,000 Gibbs samples. Afterward, GBV-RRM were calculated by multiplying the final estimated random regression coefficients for direct genetic effects (matrix $\mathbf{A_d}$ of dimension 20,176 × 4) with Legendre polynomials coefficients

$$\mathbf{L} = \left[\sqrt{\tfrac{1}{2}}, \sqrt{\tfrac{3}{2}}t_x, \sqrt{\tfrac{5}{2}}\left(\tfrac{3}{2}t_x^2 - \tfrac{1}{2}\right), \sqrt{\tfrac{7}{2}}\left(\tfrac{5}{2}t_x^3 - \tfrac{3}{2}t_x\right)\right],$$

$$t_x = -1 + 2\frac{\left(t_l - 0\right)}{24 - 0},$$

and $t_l$ from 0 to 24 mo, implying the calculation of GBV-RRM via $\mathbf{A_dL'}$. Hence, GBV-RRM were available for all animals as included in the pedigree file, with 25 monthly GBV from age 0 to 24 mo.

A detailed overview addressing all models 1 to 5 is given in Table 2.

## Accuracies of Genomic Growth Curves

We focused on 3 different evaluation strategies (Figure 2). First, we considered the 620 genotyped female cattle and estimated their GBV (application of model 3) for the pseudo-phenotypes. Afterward, the GBV served as input parameters for the calculation of individual genomic growth curves using the respective NLINM. The genomic growth curves for the 620 cattle were correlated with GBV-RRM from model 5. Alter-

natively, in a second evaluation strategy, GBV for the growth curve parameters (also solutions from model 3) considering the 43 genotyped sires with more than 20 phenotyped daughters, were used for genomic growth curve modeling via NLINM. The correlations between predicted genomic growth curves of the 43 sires and GBV-RRM for the same sires from model 5 were defined as prediction accuracies for sires.

In a third evaluation approach, we focused on a 5-fold cross-validation considering the 620 genotyped female cattle. The females were randomly assigned into 5 groups. For the creation of validation sets, pseudo-phenotypes of cattle in groups 1 to 5 were masked consecutively, and the cattle from the remaining 4 groups represented the training sets. Hence, for the estimation of GBV, pseudo-phenotypes of growth curve parameters from training set animals (496 cattle) were used as input data for model 3. The predicted GBV of the 124 nonphenotyped female cattle in validation sets were input for the respective NLINM to calculate the 3 genomic growth curves. The genomic growth curves for the 124 cattle were correlated with the GBV-RRM of the same animals. The average correlation from 5 validation sets represented accuracies of prediction from 5-fold cross-validations.

The correlations between genomic growth curves and GBV-RRM were calculated as follows: (1) correlations between aggregated genomic growth curves for age 0 to 24 mo considering GBV of growth curve parameters and aggregated GBV-RRM from the 25 age months ($\mathbf{r_{sum}}$); (2) monthly correlations between genomic growth curves and GBV-RRM for ages 0 to 24 mo ($\mathbf{r_{mon}}$); (3) average of the 25 correlations (birth to 24 mo) from step 2 ($\mathbf{r_{mean}}$). Standard errors (SE) for the correlation coefficients were calculated via a bootstrap approach, using the "boot" function with 1,000 replicates, as implemented in the R package "boot" (Canty and Ripley, 2019).

## RESULTS AND DISCUSSION

### *Nonlinear Models and Growth Curve Parameters*

With regard to the growth curve modeling, consideration of the herd effect plus fitting an exponential residual substantially improved the model evaluation criteria (Table 3). This was the case for all 3 functions LOG, GOM, and RICH. Consequently, in the ongoing genomic analyses, pseudo-phenotypes were growth curve parameters estimated from NLINM with herd effect and exponential residual. An across-function comparison for −2LL, Akaike information criterion, and Bayesian information criterion indicated superiority of RICH over GOM and LOG. Additionally, the residuals

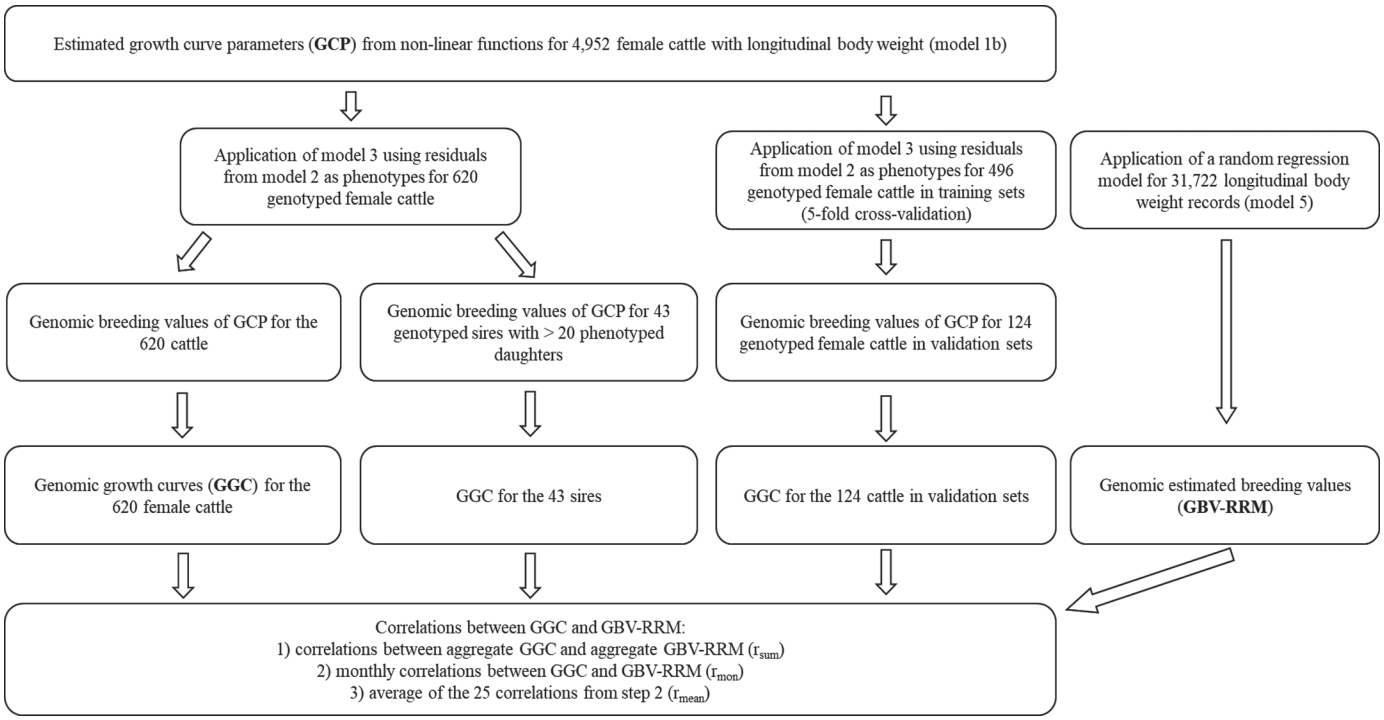


**Figure 2.** Strategies to evaluate accuracies of genomic growth curves predicted from genomic breeding values of growth curve parameters.

**Table 3.** Model evaluations for the nonlinear growth functions (models 1a and 1b) with different covariates and residuals based on −2 times maximized log-likelihood (−2LL), Akaike information criterion (AIC), and Bayesian information criterion (BIC)

| Growth function | Covariate | Residual | Evaluation criterion | | |
|---|---|---|---|---|---|
| | | | −2LL | AIC | BIC |
| Logistic | | | | | |
| | — | Homogeneous | 289,798.7 | 289,812.7 | 289,858.2 |
| | — | Exponential | 279,817.7 | 279,831.7 | 279,877.3 |
| | Herd | Homogeneous | 284,216.8 | 284,284.8 | 284,506.0 |
| | Herd | Exponential | 276,447.4 | 276,515.4 | 276,736.7 |
| Gompertz | | | | | |
| | — | Homogeneous | 282,626.9 | 282,640.9 | 282,686.5 |
| | — | Exponential | 275,008.9 | 275,022.9 | 275,068.5 |
| | Herd | Homogeneous | 278,917.5 | 278,985.5 | 279,206.7 |
| | Herd | Exponential | 272,580.3 | 272,648.3 | 272,869.5 |
| Richards | | | | | |
| | — | Homogeneous | 283,757.6 | 283,775.6 | 283,834.2 |
| | — | Exponential | 274,851.8 | 274,869.8 | 274,928.4 |
| | Herd | Homogeneous | 277,796.9 | 277,886.9 | 278,179.8 |
| | Herd | Exponential | 270,405.9 | 270,495.9 | 270,788.7 |

for the 3 NLINM ranged from 0.08 (for GOM and RICH) to 0.09 (for LOG; Table 4), again indicating the superiority of GOM and RICH over LOG. The RICH function is a generalized growth function for a variety of other functions (i.e., also for LOG and GOM) due to the possibility of modeling the shape parameter $m$ (Richards, 1959). For $m$ approaching 0, RICH can be transformed to GOM, and for $m = 1$, RICH reflects LOG (Teleken et al., 2017). For our BW data, the population value for $m$ was 0.67 (Table 4), indicating an intermediate growth curve shape between GOM and LOG. Substituting $m \to 0$, $m = 0.67$, and $m = 1$ into $W_{inflection} = \left(\frac{1}{1+m}\right)^{\frac{1}{m}} W_m$ (Goshu and Rao, 2013), inflection points for LOG, RICH, and GOM were located at 50%, 46%, and 37% of the mature weight, respectively.

The population parameter estimates for birth weight ($W_0$) were 40.17 kg (GOM), 40.49 kg (RICH), and 40.72 kg (LOG). The population mature BW ($W_m$) ranged from 436.45 kg for LOG to 571.19 kg for GOM (Table 4). The population growth rates $k$ ranged from 0.14 (GOM) to 0.31 (LOG) with very small variances. However, quite large variances were observed for $W_m$, indicating the substantial BW variations of Holstein heifers close to the first insemination date.

Vázquez et al. (2012) compared the von Bertalanffy, Weibull (Seber and Wild, 1989), modified Hill (López et al., 2000), LOG, and GOM functions, and they identified superiority of the von Bertalanffy function for growth curve modeling of 6 dairy cattle breeds (Ayrshire, Jersey, Guernsey, Holstein, Charolais, and French Friesian). Teleken et al. (2017) applied RICH to modeling growth curves of Holstein bulls and identified an $m$ value of −0.22 (indicator for a von Bertalanffy function). Beltrán et al. (1992) compared Brody with RICH in 2 lines of Angus beef cows, and they suggested the application of specific functions for specific age stages. Koskan and Ozkaya (2014) made similar conclusions for modeling growth curves of Holstein calves. Hence, due to the flexible function parameter m, we generally suggest RICH applications for nonlinear growth curve modeling based on BW data.

The growth curves for 1 cow with 4 observations and 1 animal with 19 observations are plotted in Figure 3A and 3B, respectively. For only a few BW measure-

**Table 4.** Estimated population growth curve parameters from model 1b (as described in Table 2) with herd effect and exponential residual

| Growth function | Growth curve parameters[1] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $W_0$ | $W_m$ | $k$ | $m$ | $\sigma_e$ | $\sigma^2_{W_0}$ | $\sigma^2_{W_m}$ | $\sigma^2_k \times 10^4$ | $\sigma^2_m$ |
| Logistic | 40.72 | 436.45 | 0.31 | — | 0.09 | 10.18 | 381.21 | 4.79 | — |
| Gompertz | 40.17 | 571.19 | 0.14 | — | 0.08 | 11.75 | 437.13 | 0.71 | — |
| Richards | 40.49 | 452.45 | 0.25 | 0.67 | 0.08 | 11.30 | 602.30 | 0.61 | 0.01 |

[1] $W_0$ = birth weight; $W_m$ = mature BW; $k$ = constant growth rate; $m$ = shape parameter; $\sigma_e$ = residual standard deviation; $\sigma^2_{W_0}$ = variance of birth weight; $\sigma^2_{W_m}$ = variance of mature BW; $\sigma^2_k$ = variance of growth rate; $\sigma^2_m$ = variance of shape parameter.

ments during growth, LOG underestimated BW in the period from 18 to 24 mo of age. In contrast to LOG, growth curves predicted from GOM and RICH showed an almost linear positive slope during the same measuring period. The observed differences between GOM and RICH with LOG might be due to the variations in the estimations for $k$ and $W_m$. More specifically, $k$ was 0.32 for LOG, 0.14 for GOM, and 0.16 for RICH, and $W_m$ was 397.77 kg for LOG, 525.44 kg for GOM, and 490.03 kg for RICH. For a denser BW structure (Figure 3B) with more BW observations, LOG and RICH generated similar growth curves. Furthermore, only minor differences between GOM curve predictions and real BW observations were observed in the interval from 22 to 24 mo of age.

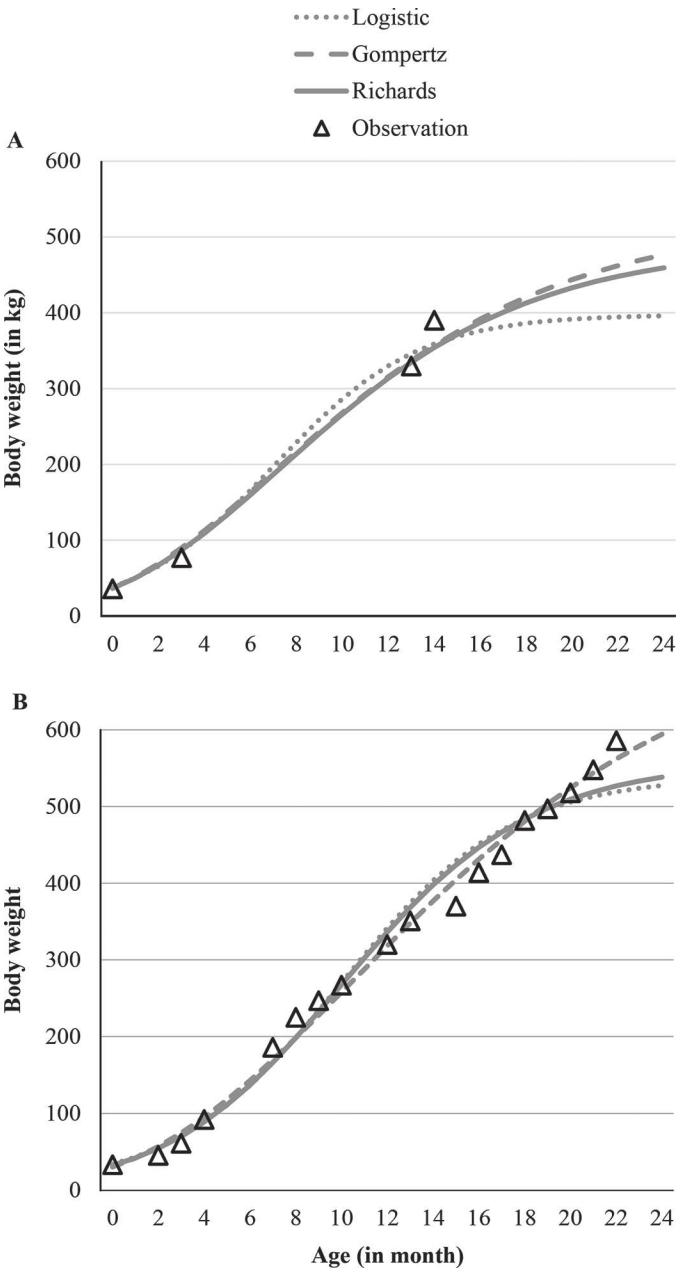


**Figure 3.** Growth curves for animals with 4 (A) and 19 (B) repeated BW measurements estimated from model 1b.

### Variance Components of Growth Curve Parameters

Variance components and genetic parameters for the 10 parameters estimated from the genomic models (model 3) with different similarity matrices are listed in Table 5. For the genomic relationship matrices **G1** and **G2**, $\sigma_g^2$ in relation to $\sigma_t^2$ are the heritabilities of the growth parameters. Heritabilities for $W_0$ from LOG and GOM and considering **G1** and **G2** were larger than 0.40. From RICH, the heritabilities for $W_0$ were 0.35 (**G1**) and 0.37 (**G2**). Heritabilities for $W_m$ in the range from 0.32 to 0.39 were slightly smaller than heritabilities for $W_0$. The moderate to high heritabilities for the growth curve parameters $W_0$ and $W_m$ confirm previous estimates based on "conventional" modeling approaches considering pedigree relationships. For birth weight in Holstein cattle, heritabilities ranged from 0.26 (Johanson et al., 2011) to 0.47 (Yin and König, 2018). For mature weight in Angus beef cattle, Kaps et al. (1999) estimated heritabilities (based on Brody curve predictions) in the range from 0.44 (estimate from a single-trait model) to 0.52 (estimate from a bivariate model). Meyer (1995) used GOM and estimated a heritability for mature weight of 0.49. Our $W_m$ heritabilities of 0.32 (**G1** and RICH) and 0.39 (**G2** and GOM) are in agreement with the heritabilities for mature weight from growth curves using lifetime BW records in 5 cattle breeds (Johnson et al., 1990) and for live weight of lactating Holstein cows (Lassen and Løvendahl, 2016). For the growth rate $k$ from GOM applications in Hereford cattle, Meyer (1995) reported heritabilities ranging from 0.37 to 0.48. Equivalent heritabilities (0.38 to 0.46) were estimated in the present study. However, in the study by Meyer (1995), values for $k$ were very small, with $k < 0.005$. In the present study, $k$ ranged from 0.01 to 0.36. Substantially lower heritabilities (<0.10) for $k$ were estimated when applying the von Bertalanffy function to modeling growth curves in 2 beef cattle breeds (Oliveira et al., 1994; Forni et al., 2007). Also for the shape parameter m, heritabilities were moderate: 0.29 (**G1**) and 0.31 (**G2**). As far as we know, this is a first study estimating heritabilities for the shape parameter (here based on RICH predictions). However, as an approximation, inflection points also describe the

**Table 5.** Variance components and variance ratios for the parameters of the growth functions from model 3 considering different similarity matrices[1]

| Similarity matrix | Growth function | Parameter | Variance component | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | $\sigma_g^2$ | $\sigma_e^2$ | $\sigma_t^2$ | $\sigma_g^2/\sigma_t^2$ | SE | $h_c^2$ |
| **G1** | Logistic | $W_0$ | 2.60 | 3.66 | 6.26 | 0.42 | 0.10 | 0.42 |
| | | $W_m$ | 38.51 | 71.74 | 110.25 | 0.35 | 0.09 | 0.35 |
| | | $k \times 10^4$ | 0.93 | 1.58 | 2.51 | 0.37 | 0.09 | 0.37 |
| | Gompertz | $W_0$ | 3.06 | 4.60 | 7.66 | 0.40 | 0.10 | 0.40 |
| | | $W_m$ | 23.77 | 39.76 | 63.53 | 0.37 | 0.09 | 0.38 |
| | | $k \times 10^4$ | 0.17 | 0.27 | 0.44 | 0.40 | 0.09 | 0.40 |
| | Richards | $W_0$ | 2.61 | 4.80 | 7.41 | 0.35 | 0.09 | 0.35 |
| | | $W_m$ | 76.46 | 164.49 | 240.96 | 0.32 | 0.08 | 0.32 |
| | | $k \times 10^4$ | 0.04 | 0.04 | 0.08 | 0.46 | 0.05 | 0.47 |
| | | $m \times 10^4$ | 13.39 | 32.05 | 45.44 | 0.29 | 0.09 | 0.30 |
| **G2** | Logistic | $W_0$ | 2.70 | 3.55 | 6.25 | 0.43 | 0.10 | 0.44 |
| | | $W_m$ | 39.32 | 71.00 | 110.32 | 0.36 | 0.09 | 0.36 |
| | | $k \times 10^4$ | 1.01 | 1.51 | 2.51 | 0.40 | 0.10 | 0.40 |
| | Gompertz | $W_0$ | 3.18 | 4.48 | 7.65 | 0.41 | 0.10 | 0.42 |
| | | $W_m$ | 24.70 | 38.93 | 63.63 | 0.39 | 0.09 | 0.39 |
| | | $k \times 10^4$ | 0.19 | 0.25 | 0.44 | 0.42 | 0.10 | 0.43 |
| | Richards | $W_0$ | 2.71 | 4.69 | 7.40 | 0.37 | 0.10 | 0.37 |
| | | $W_m$ | 79.27 | 161.75 | 241.02 | 0.33 | 0.09 | 0.33 |
| | | $k \times 10^4$ | 0.02 | 0.04 | 0.06 | 0.38 | 0.04 | 0.38 |
| | | $m \times 10^4$ | 14.09 | 31.36 | 45.45 | 0.31 | 0.10 | 0.31 |
| **K1** | Logistic | $W_0$ | 13.87 | 2.95 | 16.83 | 0.82 | 0.07 | 0.53 |
| | | $W_m$ | 217.93 | 58.72 | 276.65 | 0.79 | 0.08 | 0.47 |
| | | $k \times 10^4$ | 5.35 | 1.23 | 6.58 | 0.81 | 0.07 | 0.51 |
| | Gompertz | $W_0$ | 16.27 | 3.78 | 20.04 | 0.81 | 0.08 | 0.51 |
| | | $W_m$ | 127.16 | 33.26 | 160.42 | 0.79 | 0.07 | 0.48 |
| | | $k \times 10^4$ | 0.97 | 0.21 | 1.18 | 0.82 | 0.07 | 0.52 |
| | Richards | $W_0$ | 13.49 | 4.16 | 17.65 | 0.76 | 0.09 | 0.44 |
| | | $W_m$ | 421.32 | 140.74 | 562.06 | 0.75 | 0.08 | 0.42 |
| | | $k \times 10^4$ | 0.22 | 0.05 | 0.27 | 0.81 | 0.07 | 0.50 |
| | | $m \times 10^4$ | 72.81 | 28.20 | 101.01 | 0.72 | 0.10 | 0.38 |
| **K2** | Logistic | $W_0$ | 11.35 | 2.77 | 14.13 | 0.80 | 0.09 | 0.56 |
| | | $W_m$ | 194.20 | 51.26 | 245.47 | 0.79 | 0.08 | 0.54 |
| | | $k \times 10^4$ | 4.32 | 1.18 | 5.49 | 0.79 | 0.09 | 0.53 |
| | Gompertz | $W_0$ | 13.60 | 3.48 | 17.09 | 0.80 | 0.09 | 0.54 |
| | | $W_m$ | 106.83 | 30.81 | 137.64 | 0.78 | 0.08 | 0.51 |
| | | $k \times 10^4$ | 0.79 | 0.20 | 0.99 | 0.80 | 0.08 | 0.55 |
| | Richards | $W_0$ | 11.36 | 3.89 | 15.25 | 0.74 | 0.10 | 0.47 |
| | | $W_m$ | 363.38 | 130.09 | 493.47 | 0.74 | 0.09 | 0.46 |
| | | $k \times 10^4$ | 0.18 | 0.05 | 0.23 | 0.79 | 0.08 | 0.54 |
| | | $m \times 10^4$ | 58.77 | 27.49 | 86.25 | 0.68 | 0.12 | 0.40 |
| **K3** | Logistic | $W_0$ | 6.12 | 1.85 | 7.96 | 0.77 | 0.14 | 0.70 |
| | | $W_m$ | 104.25 | 35.98 | 140.23 | 0.74 | 0.14 | 0.67 |
| | | $k \times 10^4$ | 2.40 | 0.78 | 3.18 | 0.75 | 0.14 | 0.69 |
| | Gompertz | $W_0$ | 7.28 | 2.41 | 9.69 | 0.75 | 0.15 | 0.68 |
| | | $W_m$ | 63.01 | 18.46 | 81.47 | 0.77 | 0.14 | 0.71 |
| | | $k \times 10^4$ | 0.44 | 0.12 | 0.57 | 0.78 | 0.14 | 0.72 |
| | Richards | $W_0$ | 6.20 | 2.91 | 9.11 | 0.68 | 0.15 | 0.60 |
| | | $W_m$ | 205.03 | 94.27 | 299.30 | 0.69 | 0.14 | 0.61 |
| | | $k \times 10^4$ | 0.10 | 0.03 | 0.13 | 0.76 | 0.13 | 0.69 |
| | | $m \times 10^4$ | 31.86 | 22.61 | 54.47 | 0.58 | 0.16 | 0.50 |

[1]**G1** = genomic relationship matrix according to VanRaden, 2008; **G2** = genomic relationship matrix according to Amin et al., 2007; **K1** = kernel matrix according to Wu et al., 2011; **K2** = kernel matrix according to González-Recio et al., 2008; **K3** = Gaussian kernel; $\sigma_g^2$ = variance explained by the similarity matrix; $\sigma_e^2$ = residual variance; $\sigma_t^2$ = total variance; $\sigma_g^2/\sigma_t^2$ = variance explained by the similarity matrix in relation to the total variance; SE = standard error of $\sigma_g^2/\sigma_t^2$; $h_c^2$ = corrected heritability according to Legarra (2016); $W_0$ = birth weight; $W_m$ = mature BW; $k$ = constant growth rate; $m$ = shape parameter.

shape of growth curves. Heritabilities for the inflection points in chicken populations were 0.25 (Manjula et al., 2018) and 0.50 (Grossman and Bohren, 1985).

In comparison with **G1** and **G2**, most of the variance components increased when considering the kernels **K1**, **K2**, or **K3**. The substantially larger variances

**Table 6.** Heritabilities (bold and diagonal), genetic (above diagonal) and phenotypic correlations (below diagonal) between growth curve parameters ($W_0$ = birth weight; $W_m$ = mature BW; $k$ = constant growth rate; $m$ = shape parameter) from model 4 using a relationship matrix combining pedigree and genomic information (**H** matrix); SE are given below the parameter estimates

| Growth function | Parameter | Logistic | | | Gompertz | | | Richards | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $W_0$ | $W_m$ | $k$ | $W_0$ | $W_m$ | $k$ | $W_0$ | $W_m$ | $k$ | $m$ |
| Logistic | $W_0$ | **0.42** | 0.33 | 0.49 | 0.99 | 0.35 | 0.48 | 0.99 | 0.39 | 0.36 | −0.39 |
| | | 0.04 | 0.08 | 0.07 | 0.00 | 0.08 | 0.07 | 0.00 | 0.08 | 0.08 | 0.08 |
| | $W_m$ | 0.07 | **0.30** | 0.69 | 0.36 | 0.95 | 0.81 | 0.32 | 0.96 | 0.86 | −0.56 |
| | | 0.02 | 0.04 | 0.06 | 0.08 | 0.01 | 0.04 | 0.08 | 0.01 | 0.04 | 0.08 |
| | $k$ | 0.27 | 0.44 | **0.25** | 0.47 | 0.78 | 0.97 | 0.41 | 0.73 | 0.91 | −0.95 |
| | | 0.01 | 0.01 | 0.03 | 0.07 | 0.05 | 0.01 | 0.07 | 0.06 | 0.02 | 0.01 |
| Gompertz | $W_0$ | 0.98 | 0.09 | 0.24 | **0.43** | 0.36 | 0.47 | 1.00 | 0.40 | 0.36 | −0.35 |
| | | 0.00 | 0.02 | 0.01 | 0.04 | 0.08 | 0.07 | 0.00 | 0.08 | 0.08 | 0.08 |
| | $W_m$ | 0.11 | 0.89 | 0.57 | 0.12 | **0.31** | 0.88 | 0.31 | 0.95 | 0.92 | −0.67 |
| | | 0.02 | 0.00 | 0.01 | 0.02 | 0.04 | 0.03 | 0.08 | 0.02 | 0.02 | 0.07 |
| | $k$ | 0.28 | 0.60 | 0.96 | 0.26 | 0.71 | **0.25** | 0.41 | 0.84 | 0.94 | −0.90 |
| | | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | 0.02 | 0.07 | 0.04 | 0.01 | 0.02 |
| Richards | $W_0$ | 0.97 | 0.10 | 0.19 | 0.98 | 0.11 | 0.22 | **0.43** | 0.37 | 0.31 | −0.29 |
| | | 0.00 | 0.02 | 0.02 | 0.00 | 0.02 | 0.02 | 0.04 | 0.08 | 0.08 | 0.08 |
| | $W_m$ | 0.08 | 0.91 | 0.51 | 0.09 | 0.83 | 0.66 | 0.10 | **0.28** | 0.84 | −0.60 |
| | | 0.02 | 0.00 | 0.01 | 0.02 | 0.00 | 0.01 | 0.02 | 0.04 | 0.04 | 0.08 |
| | $k$ | 0.18 | 0.63 | 0.88 | 0.17 | 0.73 | 0.90 | 0.13 | 0.62 | **0.32** | −0.88 |
| | | 0.02 | 0.01 | 0.00 | 0.02 | 0.01 | 0.00 | 0.02 | 0.01 | 0.03 | 0.03 |
| | $m$ | −0.30 | −0.25 | −0.91 | −0.26 | −0.39 | −0.85 | −0.20 | −0.29 | −0.75 | **0.22** |
| | | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | 0.00 | 0.02 | 0.01 | 0.01 | 0.03 |

linked to the kernel and smaller residuals variances contributed to an increase of the variance ratio $\sigma_g^2/\sigma_t^2$. The variance components were further transformed into comparable scales by scaling the variances linked to the similarity matrices with $D_k$, where $D_k = \overline{\text{diag}(\mathbf{K})} - \bar{\mathbf{K}}$ (Legarra, 2016). In our study, $D_k$ was 1.01 for **G1** and **G2**, 0.24 for **K1**, 0.30 for **K2**, and 0.71 for **K3**. Corrected heritabilities from **K1** and **K2** were larger than from **G1** and **G2**. When setting **K3** as relationship matrix, the corrected heritabilities increased to 0.50 for the shape parameter and to 0.72 for the growth rate. However, SE for the variance ratios from **K3** were quite large (Table 5).

The heritabilities from the single-trait model 4 as well as genetic ($\mathbf{r_g}$) and phenotypic correlations ($\mathbf{r_p}$; from the series of bivariate runs using model 4) among growth curve parameters are given in Table 6. Heritabilities for growth curve parameters from the **H** matrix approach were identical or slightly smaller compared with the respective parameter heritabilities based on **G1** or **G2** modeling. However, compared with the **H** matrix estimates, heritabilities and variance ratios from **G1** and **G2** approaches had larger SE, probably due to the different numbers of animals with pseudo-phenotypes (4,952 cattle in model 4, and 620 cattle in model 3). Genetic correlations between the same growth curve parameters from different NLINM were larger than 0.90: 0.99 between $W_0$ from LOG with $W_0$ from GOM or RICH. The corresponding phenotypic correlations were larger than 0.82. Birth weight ($W_0$) was genetically moderately correlated with growth rate (k) and with mature weight ($W_m$; from 0.33 to 0.49). Corresponding phenotypic correlations ranged from 0.07 to 0.28. The large correlations between $k$ and $W_m$ (0.69 to 0.88 for $r_g$, and 0.44 to 0.73 for $r_p$) indicate the strong influence of growth rate on mature weight phenotypically and genetically. Accordingly, a pronounced favorable genetic correlation between $k$ and $W_m$ (0.82) was reported for predictions from the von Bertalanffy function in Nelore cattle (Forni et al., 2007). The growth rate $k$ was genetically negatively correlated with the shape parameter $m$ ($r_g$ from −0.88 to −0.95). Also for all remaining curve parameters, genetic correlations with $m$ were negative. The negative correlations indicate that increasing $W_0$, $W_m$, and $k$ influences the shape of growth curves from RICH by decreasing the percentages of mature weight at inflection points. Standard errors for correlation estimates were quite small, between 0.01 and 0.08 for $r_g$ and generally lower than 0.02 for $r_p$.

## Accuracies of Genomic Growth Curves for Animals with Phenotypes

The correlations between genomic growth curves based on GBV of growth curve parameters and respective GBV-RRM for all genotyped female cattle are listed in Table 7. For $r_{sum}$, the accuracies ranged from 0.75 to 0.79 with corresponding SE from 0.01 to 0.02. Accuracies were largest when combining estimated

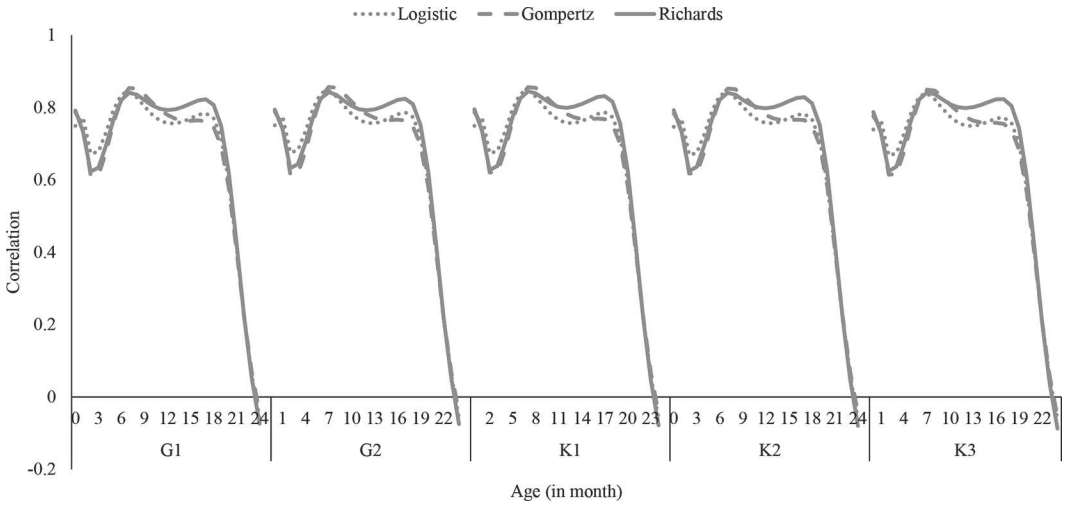


**Figure 4.** Correlations between genomic growth curves (model 3) and respective genomic breeding values from the random regression model per age month (model 5). **G1** = genomic relationship matrix according to VanRaden, 2008; **G2** = genomic relationship matrix according to Amin et al., 2007; **K1** = kernel matrix according to Wu et al., 2011; **K2** = kernel matrix according to González-Recio et al., 2008; **K3** = Gaussian kernel.

pseudo-phenotypes from RICH with **G1**, **G2**, **K1**, or **K2**. RICH always performed better than LOG or GOM, and, throughout, the lowest accuracies were identified for LOG. The increase in accuracies for RICH over LOG ranged from 0.02 (for **G2** and **K1**) to 0.03 (for **G1**, **K2** and **K3**), reflecting the phenotypic NLINM evaluations (Table 3). Apart from LOG, accuracies were maximized when modeling the kernel **K1**, but the accuracy differences for different similarity matrices were quite small.

Accuracies according to $r_{mean}$ varied between 0.65 for GOM combined with **K3** and 0.68 for RICH combined with **G1**, **G2**, **K1**, or **K2.** Generally, values for $r_{mean}$ were lower than the respective values for $r_{sum}$. Nevertheless, applications of RICH combined with **G2** or **K1** implied the largest accuracies, independent from the evaluation criterion. Standard errors for $r_{mean}$ were close to 0.02.

The detailed correlations in monthly intervals ($r_{mon}$) explain the differences between $r_{sum}$ and $r_{mean}$ (Figure 4). The correlations between genomic growth curves and GBV-RRM were larger than 0.60 from birth until the age of 20 mo. However, probably due to the low data coverage in the period from 21 to 24 mo, the accuracies substantially decreased from approximately 0.60 to −0.05 at the end of the time scale. Accordingly, the accuracy for $r_{mean}$ was quite large (0.77 for **G1**) when excluding correlations beyond 21 mo. The pattern of accuracies along the growth trajectory was very similar

**Table 7.** Correlations between genomic growth curves (model 3) and genomic breeding values (GBV) from the random regression model (model 5) considering genotyped female cattle with BW records (SE ranged from 0.01 to 0.02)[1]

| Similarity matrix | $r_{sum}$ | | | $r_{mean}$ | | |
|---|---|---|---|---|---|---|
| | Logistic | Gompertz | Richards | Logistic | Gompertz | Richards |
| **G1** | 0.76 | 0.78 | 0.79 | 0.67 | 0.66 | 0.68 |
| **G2** | 0.77[2] | 0.78 | 0.79 | 0.67[2] | 0.66 | 0.68 |
| **K1** | 0.77 | 0.78[2] | 0.79[2] | 0.67 | 0.67[2] | 0.68[2] |
| **K2** | 0.76 | 0.78 | 0.79 | 0.67 | 0.66 | 0.68 |
| **K3** | 0.75 | 0.77 | 0.78 | 0.66 | 0.65 | 0.67 |

[1]**G1** = genomic relationship matrix according to VanRaden, 2008; **G2** = genomic relationship matrix according to Amin et al., 2007; **K1** = kernel matrix according to Wu et al., 2011; **K2** = kernel matrix according to González-Recio et al., 2008; **K3** = Gaussian kernel; $r_{sum}$ = correlation between the sum of genomic growth curve breeding values and the sum of GBV from the random regression model; $r_{mean}$ = average of 25 monthly correlations between genomic growth curve breeding values and GBV from the random regression model.

[2]Highest correlation coefficient within each column.

**Table 8.** Correlations between growth curves calculated from genomic breeding values (GBV) of growth curve parameters (model 3) and GBV from the random regression model (model 5) for genotyped female cattle from a 5-fold cross-validation and for 43 genotyped sires (SE ranged from 0.07 to 0.08 for heifers and from 0.14 to 0.15 for sires)[1]

| Testing set | Similarity matrix | $r_{sum}$ | | | $r_{mean}$ | | |
|---|---|---|---|---|---|---|---|
| | | Logistic | Gompertz | Richards | Logistic | Gompertz | Richards |
| Female | **G1** | 0.40 | 0.40 | 0.41 | 0.34[2] | 0.33[2] | 0.34[2] |
| | **G2** | 0.40 | 0.40 | 0.41 | 0.34 | 0.33 | 0.34 |
| | **K1** | 0.40[2] | 0.41[2] | 0.42[2] | 0.33 | 0.33 | 0.33 |
| | **K2** | 0.39 | 0.40 | 0.41 | 0.32 | 0.32 | 0.32 |
| | **K3** | 0.40 | 0.40 | 0.41 | 0.33 | 0.32 | 0.33 |
| Sire | **G1** | 0.43 | 0.44 | 0.41 | 0.38 | 0.39 | 0.37 |
| | **G2** | 0.43 | 0.44 | 0.42 | 0.38 | 0.39 | 0.38[2] |
| | **K1** | 0.42 | 0.42 | 0.40 | 0.36 | 0.37 | 0.35 |
| | **K2** | 0.41 | 0.41 | 0.39 | 0.35 | 0.36 | 0.35 |
| | **K3** | 0.45[2] | 0.45[2] | 0.42[2] | 0.38[2] | 0.39[2] | 0.37 |

[1]**G1** = genomic relationship matrix according to VanRaden, 2008; **G2** = genomic relationship matrix according to Amin et al., 2007; **K1** = kernel matrix according to Wu et al., 2011; **K2** = kernel matrix according to González-Recio et al., 2008; **K3** = Gaussian kernel; $r_{sum}$ = correlation between sum of genomic growth curves based on GBV of growth curve parameters and the sum of GBV from the random regression model; $r_{mean}$ = average of 25 monthly correlations between genomic growth curves and GBV from the random regression model; Female = genotyped female cattle with masked pseudo-phenotypes; Sire = genotyped sires with more than 20 phenotyped daughters.

[2]Highest correlation coefficient for each nonlinear function based on 5-fold cross-validations for sires.

for the 5 different similarity matrices. Standard errors for $r_{mon}$ were smallest at age 6 to 9 mo (0.01), but were larger at birth (0.02) and at age 24 mo (0.04).

### Accuracies of Predicted Genomic Growth Curves for Animals Without Phenotypes

Genomic prediction accuracies for the female cattle from the 5-fold cross-validation and for the sires are given in Table 8 for $r_{sum}$ and $r_{mean}$. Accuracies were lower than the accuracies for female cattle with pseudo-phenotypes, such as approximately 0.40 for $r_{sum}$ (SE = 0.07 − 0.08) and approximately 0.33 for $r_{mean}$ (SE = 0.08). The lower accuracies were expected, because only accuracies for female cattle with masked pseudo-phenotypes were considered. Genomic growth curves predicted from parameters of RICH showed only minor gains in accuracies compared with GOM and LOG. With a focus on the different similarity matrices, values for $r_{sum}$ were again largest when modeling the kernel **K1**. According to $r_{mean}$, prediction accuracies were largest when modeling **G1** but showed only minor differences compared with the **G2** and **K1** modeling approaches. Standard deviations for the prediction accuracies from the 5-fold cross-validation ranged from 0.06 at birth to 0.14 at age 19 and 20 mo.

Given the fact that only influential sires with more than 20 phenotyped daughters were considered, the prediction accuracies for sires were larger than for the female cattle. The SE for the accuracies ranged from 0.14 to 0.15 for all NLINM and similarity matrix applications. However, the differences in prediction accuracies between sires and female cattle were quite small, because only 31 sires additionally had daughters with genotypes. For sires, and for both evaluation criteria $r_{sum}$ and $r_{mean}$, accuracies from GOM were slightly larger than from RICH or LOG. However, when considering the corresponding SE, differences in prediction accuracy with similarity combinations did not differ significantly. de los Campos et al. (2010b) compared the Gaussian kernel average with Bayesian ridge regression and with **G**. For protein content, Bayesian ridge regression and **G** performed better than the Gaussian kernel average, but for daughter fertility, de los Campos et al. (2010b) detected opposite results. Hence, trait specifics should be considered when selecting the most appropriate similarity matrix. Furthermore, de los Campos et al. (2010a) identified almost the same residual variances and mean squared errors from a Gaussian kernel with optimal bandwidth and from the Gaussian kernel average. In the current study, the optimal bandwidth for the Gaussian kernel **K3** was chosen according to log-likelihood values. Hence, for the sire data set evaluation, **K3** modeling was associated with the largest prediction accuracies (apart from $r_{mean}$ and RICH pseudo-phenotypes). In contrast, in genomic predictions for sires without daughter records, González-Recio et al. (2008) indicated a better predictive ability for the kernel **K2** compared with **A**, trinomial kernels, linear regressions, or 24 informative SNP in a BayesA approach.

Correlations across the growth curve in monthly intervals ($r_{mon}$) for female cattle without phenotypes (SE = 0.07 − 0.09) and for sires (SE = 0.11 − 0.16)

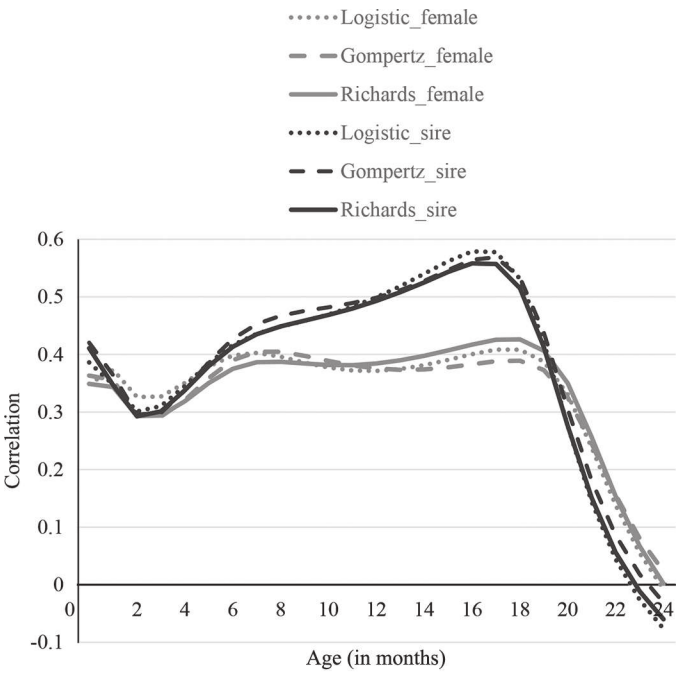


**Figure 5.** Correlations between predicted genomic growth curves based on the genomic relationship matrix (model 3) and respective genomic breeding values from the random regression model per month (model 5). Female = genotyped female cattle with masked pseudo-phenotypes; sire = genotyped sires with more than 20 phenotyped daughters.

were almost the same for different similarity matrices. Thus, only results from **G1** are plotted in Figure 5. The prediction accuracies for female cattle and sires were very similar at the extreme ends of the age scale. From birth to 5 mo of age, $r_{mon}$ varied from 0.3 and 0.4 for both validation strategies. Afterward, the correlation increased gradually to 0.58 for sires, but was 0.40 for female cattle at age 18 mo. At the end of the growth curve interval, probably due to the smaller number of animals with phenotypic records, $r_{mon}$ in both data sets sets (sires and heifers) substantially decreased, and was close to 0 at 24 mo. The shape of the prediction accuracy curves (the values for $r_{mon}$ at specific ages) was very similar for pseudo-phenotypes from LOG, GOM, and RICH applications for sires as well as for female cattle.

## CONCLUSIONS

For the modeling of phenotypic growth curves based on longitudinal BW records in dairy cattle from birth to approximate age at first calving, RICH applications with herd effects and exponential residuals contributed to superior NLINM over LOG and GOM. Heritabilities for NLINM growth curve parameters based on **G1** and **G2** modeling approaches were the same or slightly larger than the corresponding heritabilities based on the **H** matrix. Genetic correlations among the growth curve parameters $W_0$, $W_m$, and $k$ were always positive (>0.30), especially for the same growth curve parameters estimated from different NLINM (>0.90). The shape parameter $m$ in RICH was genetically negatively associated with other growth curve parameters. Accuracies of the genomic growth curves for the 620 female cattle with pseudo-phenotypes favored RICH applications over LOG and GOM, irrespective of the similarity matrices used. However, due to the large SE, differences in prediction accuracies from the different approaches considering sires with more than 20 daughters with BW records, or the results from the 5-fold cross-validations, were not significant. Generally, when focusing on animals without phenotypes, prediction accuracies were larger for sires than for female cattle, especially for specific ages in the interval from age 6 mo to 18 mo. Hence, increasing the phenotypic BW data set is more important for the increase of genomic growth curve accuracies than are function or similarity matrix specifications. For complex modeling approaches, as used in the present study based on a quite large number of phenotypes, it is also imperative to increase the number of genotyped animals.

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## ORCIDS

S. König https://orcid.org/0000-0002-1716-2511

## 2.7. Original research paper 7

**Yin**, **T.**, M. Jaeger, C. Scheper, G. Grodkowski, T. Sakowski, M. Klopčič, B. Bapst, and S. König:

Multi-breed genome-wide association studies across countries for electronically recorded behavior traits in local dual-purpose cows.

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RESEARCH ARTICLE

# Multi-breed genome-wide association studies across countries for electronically recorded behavior traits in local dual-purpose cows

**Tong Yin[1☯], Maria Jaeger[1☯], Carsten Scheper[1], Gregorz Grodkowski[2], Tomasz Sakowski[2], Marija Klopčič[3], Beat Bapst[4], Sven König[1]***

**1** Institute of Animal Breeding and Genetics, Justus-Liebig-University of Gießen, Gießen, Germany, **2** Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Jastrzębiec, Poland, **3** University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Domzale, Slovenia, **4** Genetic evaluation center, Qualitas AG, Switzerland

☯ These authors contributed equally to this work.
* sven.koenig@agrar.uni-giessen.de





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## Abstract

Basic bovine behavior is a crucial parameter influencing cattle domestication. In addition, behavior has an impact on cattle productivity, welfare and adaptation. The aim of the present study was to infer quantitative genetic and genomic mechanisms contributing to natural dual-purpose cow behavior in grazing systems. In this regard, we genotyped five dual-purpose breeds for a dense SNP marker panel from four different European countries. All cows from the across-country study were equipped with the same electronic recording devices. In this regard, we analyzed 97,049 longitudinal sensor behavior observations from 319 local dual-purpose cows for rumination, feeding, basic activity, high active, not active and ear temperature. According to the specific sensor behaviors and following a welfare protocol, we computed two different welfare indices. For genomic breed characterizations and multi-breed genome-wide association studies, sensor traits and test-day production records were merged with 35,826 SNP markers per cow. For the estimation of variance components, we used the pedigree relationship matrix and a combined similarity matrix that simultaneously included both pedigree and genotypes. Heritabilities for feeding, high active and not active were in a moderate range from 0.16 to 0.20. Estimates were very similar from both relationship matrix-modeling approaches and had quite small standard errors. Heritabilities for the remaining sensor traits (feeding, basic activity, ear temperature) and welfare indices were lower than 0.09. Five significant SNPs on chromosomes 11, 17, 27 and 29 were associated with rumination, and two different SNPs significantly influenced the sensor traits "not active" (chromosome 13) and "feeding" (chromosome 23). Gene annotation analyses inferred 22 potential candidate genes with a false discovery rate lower than 20%, mostly associated with rumination (13 genes) and feeding (8 genes). Mendelian randomization based on genomic variants (i.e., the instrumental variables) was used to infer causal inference between an exposure and an outcome. Significant regression coefficients among behavior traits indicate that all specific behavioral mechanisms contribute to similar physiological processes. The regression coefficients of rumination and feeding on milk yield were 0.10 kg/% and 0.12 kg/

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%, respectively, indicating their positive influence on dual-purpose cow productivity. Genomically, an improved welfare behavior of grazing cattle, i.e., a higher score for welfare indices, was significantly associated with increased fat and protein percentages.

## Introduction

The current fundamental interest in dairy cattle research addresses a deeper understanding of the role of genetics in phenotypic expressions of behavior traits. Behavior is an essential part of biological regulations and influences the production and welfare of farm animals. However, the underlying genetic mechanisms explaining the relationships between cattle behavior and productivity are unclear. Currently, consumer demands strongly influence animal husbandry and management decisions, e.g., a wish towards the utilization of natural and cow friendly production systems. Furthermore, there are increasing concerns, critically addressing the high yielding Holstein Friesian breed and suggesting local dual-purpose cattle as a breed alternative. Against this background, a better understanding of the genetic mechanisms of animal behavior allows for the implementation of local dual-purpose cattle selection strategies for specific environments, e.g., for specific grazing conditions. Hohenboken [1] listed behavior traits in cattle under genetic control, such as feeding and reproductive behavior, social interactions and temperament. In addition, especially in grazing systems, a proportion of variation in foraging behavior is genetically inherited [2,3]. In addition to feeding, rumination time and rumination intervals are defined as novel traits that influence milk yield and butterfat production [4]. Nevertheless, subjectively scored cattle behavior traits are low to moderate heritability traits, with heritabilities ranging from 0.01 to 0.44 [5,6]. Despite a few quantitative genetic studies based on pedigree relationship matrices, there is a gap in knowledge addressing genomic mechanisms of behavior trait expressions [4]. Dense longitudinal phenotypic data and dense single nucleotide polymorphism (SNP) marker information are required to perform genome-wide association studies (GWAS) and to unravel the genetic architectures of complex traits. Consequently, only a limited number of potential candidate genes significantly associated with cattle behavior traits were identified [7]. Alam et al. [8] detected polymorphisms of the bovine neuropeptide Y5 receptor gene (*NPY5R*), which regulates appetite and feeding behavior in beef cattle. Similar mechanisms for polymorphisms of the melanocortin 4 receptor gene (*MC4R*), i.e., influences on feed intake capacity and feeding behavior, were reported in Korean Hanwoo cattle [9]. Nevertheless, a strong environmental component influences behavior trait expressions, suggesting a detailed recording of environmental effects for a broad pattern of behavior characteristics [4].

In the process of animal husbandry system intensifications, domestication and artificial selection via specific mating plans were major driving components contributing to extensive linkage disequilibrium (LD) across the bovine genome [10,11]. Consequently, broad confidence intervals for significant SNP were identified, implying difficulties in precisely mapping potential candidate genes [10]. Raven et al. [10] hypothesized that lower levels of long-range LD across bovine breeds, and thus, a multi-breed GWAS, could accurately pinpoint the location of well-conserved functional mutations. When considering several breeds simultaneously, LD over short distances (5–10 kb for *Bos taurus*) already reached $r^2 > 0.3$ [12], while long-range LD decreased. Hence, with higher probability compared to a single-breed GWAS, a significant SNP from a multi-breed GWAS is located in close distance to a quantitative trait locus (QTL), which has an effect on the same trait across breeds. Significant across-breed SNP effects

are mainly due to LD with the QTL and are independent of pedigree relationship influences [11,13]. This phenomenon is well exploited in refining QTL regions in dogs, but the methodology only contributed to a limited number of identified potential candidate genes [14]. In detail, in the dog study, identification of QTLs was based on a single dog breed with extensive LD. In a second step, multiple dog breeds and dense SNP chips were used to precisely map causal variants [10,14]. Hence, with regard to QTLs segregating in multiple breeds, a multi-breed GWAS implies more precise mapping, while within-breed analyses contribute to improved detection power for breed-specific QTLs. Hence, a multi-breed GWAS might increase the probability of detecting older conserved mutations, but it is less efficient in identifying recently diverged mutations [10]. With the aim of inferring the causes of general and well-conserved genetic mechanisms in basic bovine behavior traits, a multi-breed GWAS seems to be a promising method.

The current study is based on SNP data from five dual-purpose cattle breeds located in Germany (DE_DSN = black and white dual-purpose cattle), Poland (PL_BS = Brown Swiss, PL_DSN = black and white dual-purpose cattle), Slovenia (Sl_BS = Brown Swiss, Sl_Si = Simmental) and Switzerland (CH_OBV = dual-purpose Original Braunvieh, CH_Si = Simmental). Genotyped cows were phenotyped based on 24 hours of continuously recorded behavior data in grazing systems. The overall hypothesis is that electronically recorded natural behavior of cows for feeding (FEED), ruminating (RUM), resting / non-active (NACT), basic activity (BACT) and high activity (HACT) and digital ear surface temperature (ET) contributes to the detection of significant SNP markers and associated potential candidate genes across the bovine genome. Additionally, for population structure analyses, we considered genotypes from the dual-purpose Red and White breed from Germany (DE_DN) and from German Holstein (DE_HF) and Slovenian Holstein (Sl_HF) subpopulations. We assume that different breeds with a similar breeding history share ancestral mutations and recombination events. Accordingly, Gutiérrez-Gil et al. [15] identified selection signatures influencing metabolic homeostasis and disease resistance across breeds with different production trait characteristics.

The present study is based on dense genomic marker data and longitudinal behavior traits from different dual-purpose cows across European country borders. Such unique data can be used i) to infer the population structure for European dual-purpose and dairy cattle breeds; ii) to estimate genetic parameters for behavior traits based on pedigree and genomic information; iii) to detect associated SNP and potential candidate genes significantly influencing cattle behavior; and iv) to infer causal trait associations.

## Results

### Population structure and breed assignment

**Principal component analyses.** When plotting the first and the second principal components (explaining 4.71% and 3.05% of the variation in genomic relationships, respectively), two distinctly diverged clusters of genetic origin were detected (Fig 1A). The Holstein lines and DSN showed obvious genetic differentiation from the other breeds (Sl_Si, Sl_BS, PL_BS, CH_OBV, and CH_Si). Depicting the first and third (explaining 2.38% of variation) principal components, three clusters were formed in a triangle-like 2-dimensional form (Fig 1B). Each cluster was positioned at the three apexes of the triangle, with the admixed populations of Sl_Si in an intermediate position. The first cluster includes DE_HF, DE_DSN, DE_DN, Sl_HF and PL_DSN; the second cluster consists of PL_BS, Sl_BS and CH_OBV; and CH_Si and Sl_Si (but in slight distance) are represented in cluster 3. The three clusters were also identified when plotting the second and third principal components (Fig 1C). However, the second principal component illustrates the diversity within the Holstein lines and DSN.

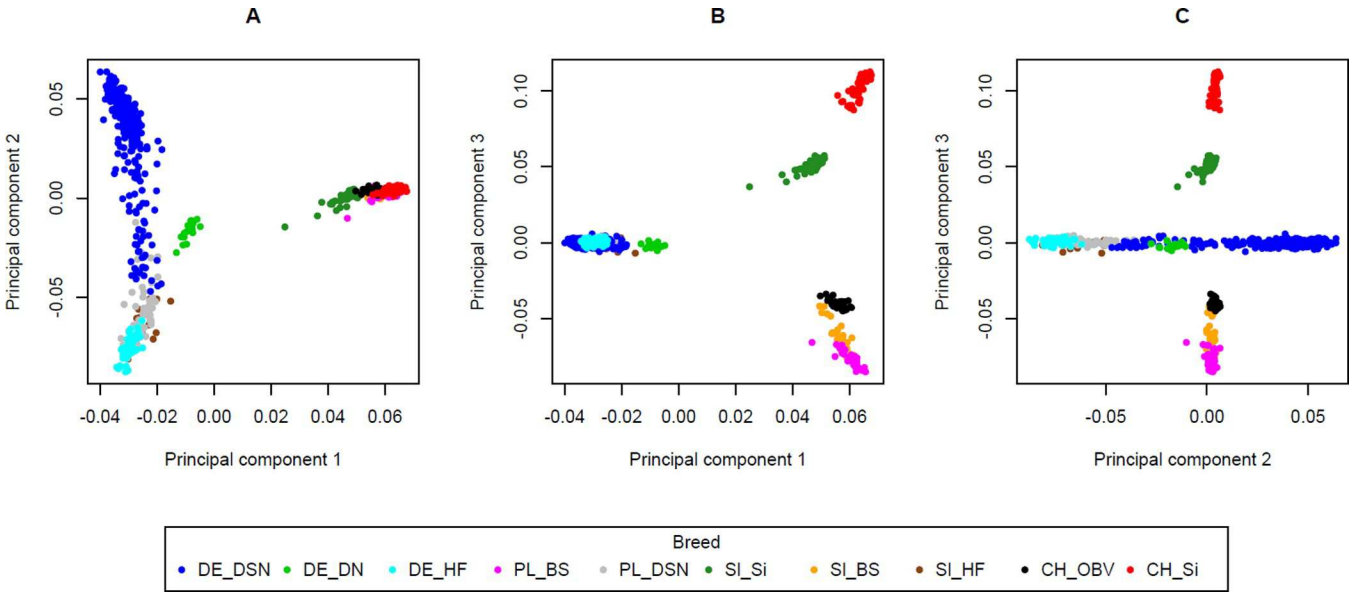


**Fig 1. Plot of principal components 1 versus 2 (A), 1 versus 3 (B) and 2 versus 3 for the genomic relationship matrix based on 615 genotyped cows.** DE_DSN = black and white dual-purpose (Germany); DE_DN = red and white dual-purpose (Germany); DE_HF = Holstein Friesian (Germany); PL_BS = Brown Swiss (Poland); PL_DSN = black and white dual-purpose (Poland); Sl_Si = Simmental (Slovenia); Sl_BS = Brown Swiss (Slovenia); Sl_HF = Holstein Friesian (Slovenia); CH_OBV = dual-purpose Original Braunvieh (Switzerland); CH_Si = Simmental (Switzerland).

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**Breed assignment.** The breed assignment (Fig 2) identified ten cattle breeds with the largest ancestry proportions from the world reference dataset in Web-Interfaced Next Generation Database (WIDDE) [16] for the populations in this study. All populations from our study shared at least 57.83% of alleles with European breeds, affirming their European origin. The predominant genetic ancestry consisted of Holstein, Hereford, French Red Pied Lowland and

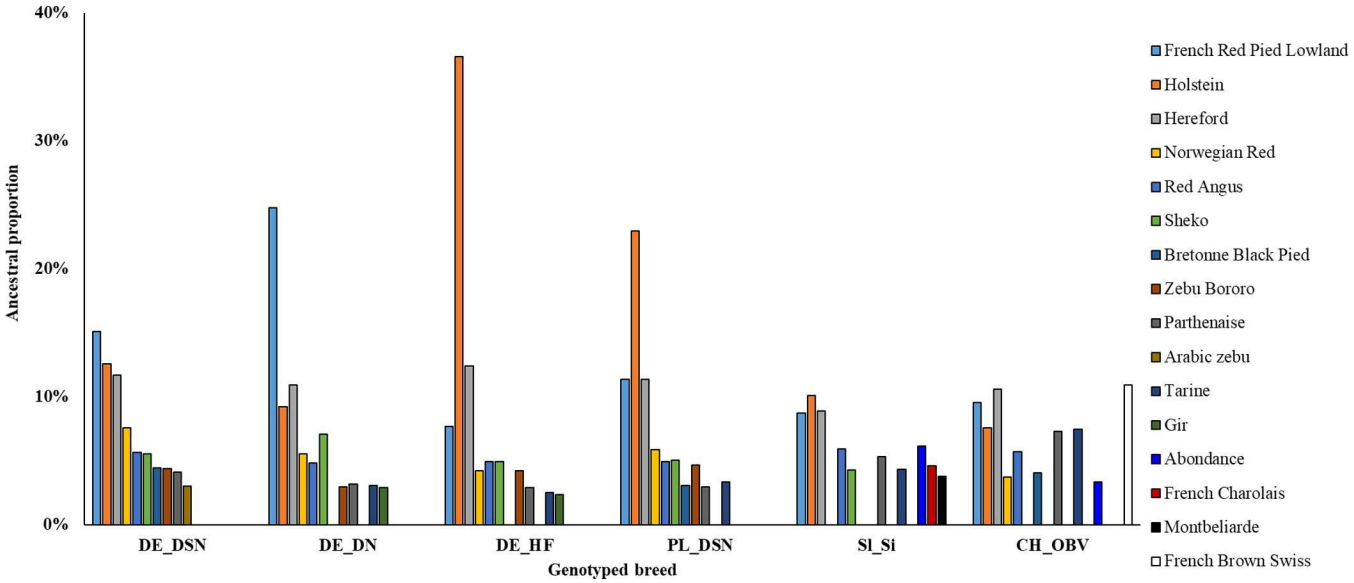


**Fig 2. Ancestry composition of the genotyped cows considering the ten reference populations in WIDDE [16].** DE_DSN = black and white dual-purpose (Germany); DE_DN = red and white dual-purpose (Germany); DE_HF = Holstein Friesian (Germany); PL_DSN = black and white dual-purpose (Poland); Sl_Si = Simmental (Slovenia); CH_OBV = dual-purpose Original Braunvieh (Switzerland).

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French Brown Swiss breeds. However, aside from European ancestors, exotic ancestral proportions from Sheko, Zebu Bororo, Gir or Arabic Zebu appeared.

## Genetic parameters for sensor traits

Heritabilities and corresponding standard errors or sensor traits were on a low to moderate level but very similar for the estimations based on either the pedigree relationship matrix (**A**) or the combined relationship matrix (**H**) matrices (Table 1). Heritabilities for FEED, HACT and NACT ranged from 0.16 to 0.20 due to moderate additive genetic and small residual variances. The low heritable traits RUM, BACT, ET, welfare index points (WEL-IP) and welfare index classes (WEL-IC) had small additive genetic variances. Repeatabilities were moderate (0.24–0.34) for sensor traits but ranged on a lower level from 0.10 to 0.13 for the two welfare indices. Standard errors of repeatabilities from the multi-breed estimation were quite small (0.01–0.02).

## Multi-breed genome-wide association study

Overall, according to the 20% false discovery rate (FDR) threshold, seven SNP markers were significantly associated with behavior traits (Table 2). One of these SNPs was also significant considering the more stringent Bonferroni threshold. Significant SNPs were located on six different chromosomes and associated with the behavior traits NACT, RUM and FEED. The most significant SNP influencing NACT is located on *Bos taurus* autosome 13 (BTA13, *P-value* = 2.36E-08) (S1 Fig). Five SNPs on BTA11, 17, 27, and 29 were significantly associated with RUM (S2 Fig), and another SNP on BTA23 was significantly associated with FEED (S3 Fig). A more significant SNP was detected for the dependent variable de-regressed proof (DRP) in comparison to the means of repeated sensor traits (MEAN). Only for NACT, the same SNP on BTA13 (Hapmap60738-rs29023086) significantly influenced both dependent variables MEAN and DRP. The inflation factors for all GWAS runs ranged from 1.00 to 1.04 for DRP, and from 0.88 to 0.99 for MEAN, indicating restricted false positives from population stratification.

The SNP coverage was examined by counting the number of SNPs in consecutive windows of 1 Mb on each chromosome. The mean SNP coverage per Mb considering the 29 autosomes was 14.2, ranging from zero to 27 SNP per Mb. At least one Mb window without a SNP was identified on ten chromosomes (BTA6, 7, 10, 12, 14, 15, 18, 21, 24 and 26).

**Table 1. Heritabilties ($h^2$) and reliabilities (r) with standard errors (SE) for sensor behavior traits.**

| | Pedigree | | | | Combined pedigree and genomic data | | | |
|---|---|---|---|---|---|---|---|---|
| **Trait** | $h^2$ | SE | r | SE | $h^2$ | SE | r | SE |
| **RUM** | 0.02 | 0.04 | 0.28 | 0.02 | 0.02 | 0.04 | 0.28 | 0.02 |
| **FEED** | 0.19 | 0.05 | 0.26 | 0.02 | 0.20 | 0.05 | 0.27 | 0.02 |
| **BACT** | 0.08 | 0.05 | 0.26 | 0.02 | 0.06 | 0.05 | 0.26 | 0.02 |
| **HACT** | 0.19 | 0.05 | 0.27 | 0.02 | 0.20 | 0.05 | 0.29 | 0.02 |
| **NACT** | 0.16 | 0.06 | 0.33 | 0.02 | 0.18 | 0.06 | 0.34 | 0.02 |
| **ET** | 0.07 | 0.04 | 0.24 | 0.02 | 0.07 | 0.04 | 0.25 | 0.02 |
| **WEL-IP** | 0.03 | 0.02 | 0.12 | 0.01 | 0.04 | 0.02 | 0.13 | 0.01 |
| **WEL-IC** | 0.03 | 0.02 | 0.10 | 0.01 | 0.04 | 0.02 | 0.10 | 0.01 |

RUM = rumination; FEED = feeding; BACT = basic active; HACT = high active; NACT = not active; ET = ear temperature; WEL-IP = welfare index point; WEL-IC = welfare index class.

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**Table 2. List of SNP markers significantly associated with sensor behavior traits (significance threshold: False discovery rate of 20%).**

| Trait | Chr. | SNP | bp | *P-value* | Method | Prop. |
|---|---|---|---|---|---|---|
| **RUM** | 11 | BTB-01638234 | 55229674 | 2.04E-05 | DRP | 2.73% |
| **RUM** | 17 | ARS-BFGL-NGS-104430 | 68187177 | 1.79E-05 | DRP | 3.38% |
| **RUM** | 27 | ARS-BFGL-NGS-13449 | 37283994 | 1.36E-05 | DRP | 3.03% |
| **RUM** | 29 | ARS-BFGL-NGS-24800 | 46014507 | 9.07E-06 | DRP | 2.91% |
| **RUM** | 29 | ARS-BFGL-NGS-81862 | 49036580 | 2.01E-05 | DRP | 3.43% |
| **FEED** | 23 | ARS-BFGL-NGS-80066 | 19834215 | 5.13E-06 | DRP | 4.41% |
| **NACT** | 13 | Hapmap60738-rs29023086 | 79178395 | 2.36E-08<br>1.08E-06 | DRP<br>MEAN | 5.51%<br>3.63% |

RUM = rumination; FEED = feeding; NACT = not active; Chr. = chromosome number; bp = base pair; DRP = de-regressed proof; MEAN = mean of observations; Prop. = proportion of phenotypic variance explained by SNP markers.

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With regard to the GWAS for fat percentage (Fat%), two significant SNPs above the Bonferroni corrected threshold on BTA14 (S4 Fig) were identified. This is the chromosomal segment for the *DGAT1* gene. Hence, the multi-breed GWAS identified the most prominent candidate gene in dairy and dual-purpose cattle.

## Potential candidate genes

Based on the *P-values* of all SNPs, i.e., GWAS output, 22 potential candidate genes were identified (S1 Table). All of the inferred potential candidate genes might play a role in the expression of bovine behavior in dual-purpose cattle populations. The 13 potential candidate genes on BAT21, 27, and 29 are associated with RUM. For RUM, one potential candidate gene (*BTBD1*) is located on BAT21, and two are on at BAT27 (*THAP1* and *RNF170*). The remaining ten potential candidate genes are located on at BAT29. Both dependent variables for RUM (DRP and MEAN) were associated with eight putative candidate genes (*RPS6KB2*, *PTPRCAP*, *CORO1B*, *GPR152*, *CABP4*, *TMEM134*, *AIP*, and *PITPNM1*). With regard to the DRP for RUM, we identified two potential candidate genes without clear biological functions on BTA29 (the novel gene *ENSBTAG00000000776* and *MRGPRG)*. The SNP Hapmap48998-BTA-104140 (*P-value* = 6.55E-06 on BAT19) suggested *PPM1E* as a potential candidate gene for BACT. With regard to the DRP of FEED, we identified seven potential candidate genes on BAT11 (*STXBP1*, *CFAP157*, *PTRH1*, *TOR2A*, *LCN8*, *LCN15* and *PPP1R26*). The two neighboring SNPs ARS-BFGL-NGS-80066 and ARS-BFGL-NGS-111955 on BTA23 indicated one putative candidate gene (*SLC25A27*) for FEED.

## Causal associations

The regression coefficients for the variety of trait associations are summarized in Table 3. The values in bold indicate significance according to FDR $< 0.05$. Behavior related to feed intake, i.e., RUM and FEED, had a significantly negative impact on behavior reflecting locomotion (BACT and HACT) and vice versa. Associations were positive between traits from the same behavior category, i.e., between FEED and RUM, and between HACT and BACT. For example, for an increase of 1% in FEED, RUM increased by 0.12%. The regression coefficient of HACT on BACT was 0.18%. Sleeping and resting (NACT) negatively influenced RUM, FEED, and HACT. When NACT was the exposure and BACT was the outcome, the slope was 0.10%. The impact of behavior traits on ET was generally weak and not significant. For example, a 1% increase in HACT was associated with an increase of 0.11 degrees Celsius for ET. Apart from HACT, behavior traits responded significantly to alterations of welfare indices because

**Table 3. Regression coefficients among sensor behavior traits and between sensor behavior traits and production traits.**

| Trait | RUM | FEED | BACT | HACT | NACT | ET | WEL-IP | WEL-IC | MY | Fat% | Pro% | SCS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RUM** | | **0.14** | **-0.40** | **-0.09** | **-0.51** | 0.02 | 0.02 | **0.00** | 0.10 | 0.00 | 0.00 | **0.05** |
| **FEED** | **0.12** | | **-0.34** | **-0.06** | **-0.48** | 0.00 | **-0.05** | **0.01** | **0.12** | 0.00 | 0.00 | 0.02 |
| **BACT** | **-0.77** | **-0.78** | | **0.18** | 0.19 | -0.01 | **0.05** | **-0.02** | -0.14 | 0.00 | 0.00 | -0.05 |
| **HACT** | **-0.51** | -0.29 | **0.59** | | **-0.78** | 0.11 | **0.07** | -0.01 | 0.04 | 0.02 | 0.01 | -0.05 |
| **NACT** | **-0.41** | **-0.59** | **0.10** | **-0.10** | | -0.01 | **-0.03** | **0.01** | **-0.14** | 0.00 | 0.00 | -0.02 |
| **WEL-IP** | **1.60** | **-0.97** | **0.80** | 0.29 | **-1.72** | **0.44** | | **-0.23** | -0.10 | 0.07 | **0.05** | 0.12 |
| **WEL-IC** | **-5.00** | 1.74 | **-4.67** | -0.61 | **7.99** | -1.44 | -3.56 | | 1.40 | **-0.57** | **-0.30** | -0.65 |

RUM = rumination; FEED = feeding; BACT = basic active; HACT = high active; NACT = not active; ET = ear temperature; WEL-IP = welfare index point; WEL-IC = welfare index class; MY = milk yield (in kg); Fat% = fat percentage; Pro% = protein percentage; SCS = somatic cell score; the bold values represent significant regression coefficients.

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behavior traits are components of the indices. An increase in feeding behavior (RUM and FEED) had favorable effects on milk yield (MY) but impaired udder health (increase of somatic cell score; SCS). A regression coefficient of -0.14 kg/% was estimated for the response of MY on NACT. Welfare indices were significantly associated with Fat% and protein percentage (Pro%).

## Discussion

### Population structure analyses

The identified breed clusters from the principal component analysis (PCA) reflect the geographical origin of the European cattle breeds. The optimization criterion for PCA is the maximization of variation in the genomic relationship matrix considering the first principal components [17], which also contribute to geographic differentiation. The PCA clearly differentiated between the Holstein lines and DSN with the Simmental and Brown Swiss breeds (Fig 1A), regardless of geographic distance. Hence, these breeds have different ancestors and do not share the same founder alleles. Due to their pronounced genetic relationships, Sl_HF, PL_DSN, DE_HF, DE_DSN, and DE_DN were allocated to the same cluster. The other two distinct clusters represent Simmental and Brown Swiss breeds (Fig 1B and 1C). The origin of genotypes certainly contributed to cluster formation. Furthermore, breed-specific breeding goals or country and farm specificities influenced breed differentiations [18]. Interestingly, only the second principal component presented genetic diversity within the cluster containing Holstein lines and DSN. Because of different breeding goal definitions [19], commercial Holstein lines (DE_HF and Sl_HF) were separated from black and white dual-purpose cattle (DE_DSN and DE_DN) during selection. The PL_DSN, reflecting an intermediate breeding goal "between" classical dual-purpose cattle and modern HF, is consequently grouped between Holstein lines and DSN. Nevertheless, DSN is the dominant founder population for modern HF [20], and similar breeding schemes and an identical herdbook were used before officially separating the two breeds in 1997 [21]. Differentiations between the Holstein lines and DSN underline the footprints of artificial selection in the last two decades [22]. Although DE_DN and DE_DSN are dual-purpose breeds, they share ancestors with Holstein Friesian cattle more than 50 years ago, explaining their rather close relationship. As a consequence, the PCA results reflect these breed origins and separate endogamous breeding units for Holstein, DSN, Brown Swiss and Simmental cattle, emphasizing the historical 'genetic isolation' by the absence of admixture.

Breed assignment results gave deeper insights into the pattern of genetic diversity and principles of historical evolutionary processes in dual-purpose cattle populations. All breeds depicted at least 57.83% of ancestral allele proportion to European cattle breeds, affirming their European origin. Nevertheless, for the European dual-purpose cattle genotypes, exotic ancestral allele proportions from Sheko, Zebu Bororo, Gir or Arabic Zebu were identified. Despite the fact that cattle are ascribed to two major geographic types, i.e., taurine (humpless-European, African, Asian) and indicine (humped- South Asian, East African), the same ancestors were identified more than 250,000 years ago [12]. Ancient genetic ties to a common ancestor as well as interbreeding [12] explain a proportion of up to 7.09% shared alleles between Sheko with DE_DSN, DE_DN, DE_HF, PL_DSN and Sl_Si. In this regard, the Bovine HapMap Consortium [12] specified five unique endogamous breeding units (Holstein, Jersey, Hereford, Romagnola, and Guernsey) and one closed endogamous breeding unit (Brown Swiss, Norwegian Red, Limousin, Charolais, and Piedmontese) for ten European breeds. Furthermore, they [12] identified indicine and taurine crosses, such as Beefmaster, Santa Gertrudis and Sheko. Accordingly, in the present study, low proportions of common ancestry between populations from our study and indicine breeds were identified.

Early breeders who spread from the Fertile Crescent towards North-West Europe used two different migration routes [23]. One route to northern Europe followed the Balkan rivers (Danubian route) to Germany and the Netherlands, while the second route (Mediterranean route) to western Europe (Italy, Spain and France) crossed the Mediterranean Sea [23]. During these migration waves, potential interbreeding between wild European aurochs and already domesticated populations explain the exotic breed footprints within the European bovine genome [17]. These findings are in agreement with the known shared ancestry between Holstein and Norwegian Red [12]. Consequently, we also detected genetic relations between DE_DSN, DE_DN, DE_HF and PL_DSN with Norwegian Red. Gautier et al. [17] affirmed the Northern European origin of Angus, Red Angus, French and American Holstein, French Red Pied Lowland and Norwegian Red cattle via Reynolds genetic distances (computation based on allele frequencies at 44,706 SNP loci). Hence, these results [17] support the identified ancestry proportions, as illustrated in Fig 2. Close genetic proximity between French Red Pied Lowland with DE_DSN, DE_DN, and DE_HF is due to the Red Pied Lowland's recent derivation from Red Holstein and Meuse-Rhin-Yssel breeds [17]. Relatively high proportions of ancestry between Hereford with Sl_Si (8.87%), CH_OBV (10.58%), DE_DN (10.92%), PL_DSN (11.37%), DE_DSN (11.69%) and DE_HF (12.39%) were identified. Accordingly, Gautier et al. [17] allocated Hereford, Holstein and Brown Swiss to one major cluster. The genetic influence of Hereford on Holstein, DSN, Brown Swiss and Simmental genomes is due to historical interbreeding events [24], which occurred before the establishment of the Hereford breed herd in 1846 [25].

The PCA results as well as the breed assignment analyses indicate the European origin of dairy (DE_HF) and dual-purpose breeds (DE_DSN, DE_DN, PL_DSN, Sl_Si, and CH_OBV) and reflect selection according to geographic and breeding goal characteristics. The evolutionary formative events contributed to the establishment of different genetic variants in cattle breeds in different regions. Moreover, they influenced the differentiation of allele frequencies among populations [12] and the associations between phenotypes and genotypes.

## Genetic parameters for sensor behavior traits

Apart from BACT, genetic parameter estimates from the pedigree-based approach (**A** matrix) were very similar or slightly smaller compared to the **H** matrix approach (i.e., additionally considering genomic marker data). This result is in agreement with other studies focusing on

genetic parameter estimations based on different genetic relationship matrices [26,27]. Basic dairy cattle habits (e.g., HACT, NACT and FEED) underlie moderate genetic control. For RUM, BACT, ET, WEL-IP and WEL-IC, the small heritabilities indicate pronounced environmental influence and challenges for genetic improvements. Nevertheless, the recording technique might also explain the lower heritabilities for sensor-recorded RUM and BACT. As a recording alternative, microphone-monitored rumination time contributed to heritability estimates for RUM in the range from 0.14 to 0.44 in Holstein cows [6]. Another reason for the smaller RUM heritability in the current study addresses characteristic differences in the production system. In grazing systems, with a higher percentage of fresh fibrous grass in the feeding ratio, rumination mechanisms might differ from total mixed rations fed in indoor systems. Hence, the genetic mechanisms for rumination might differ and influence additive genetic variances. A strong impact of food characteristics on rumination time was identified in previous studies, e.g., the influence of forage neutral detergent fiber [28], physical effective fiber [29], or long-particle alfalfa silage [30]. Nevertheless, rumination time is an interesting trait for genomic selection because of the moderate to strong association with feed efficiency [6,31]. Feeding costs are the dominant cost component in dairy and dual-purpose cattle farming systems [32]. Consequently, the selection of RUM also contributes to high feed efficiency and profitable milk production [33].

The heritability for FEED behavior is in agreement with estimates from other studies using alternative recording techniques. Løvendahl and Munksgaard [5] estimated a heritability of 0.20 for pooled eating time (hour/day) considering early and late lactation stages. Eating time was recorded via focal scanning in batches at 10-minute intervals for 24 hours. Robinson and Oddy [34] reported a heritability of 0.36 for time spent feeding, measured in automatic feeder pens. Hence, feeding time has a moderate genetic component, but the open question addresses the optimal breeding and selection strategy. A breeding goal with a focus on increasing feeding time (FEED) implies an antagonistic impact on other types of behavior, e.g., reduced lying time (NACT) [35,36].

Heritabilities for daily BACT reflect estimates based on accelerometer recordings (0.03–0.12) [37,38]. Schöpke and Weigel [37] considered 1,171 postpartum HF cows with at least 100 days of consecutive accelerometer measurements, and the HACT accelerometer heritabilities support the HACT sensor heritabilties from our study. Furthermore, in agreement with our results, variance components and heritabilities were different for different levels of activity, i.e., during nonestrus periods in the range of 0.03–0.05 and 0.12 during estrus [37]. Coincidently, in our study, heritabilities for HACT were larger than for other behavior activities. Nevertheless, the NACT heritability from the cows in the outdoor grazing system was larger than the heritability estimates of dairy cattle for lying time indoors (0.01) [5]. Even in humans, genetic parameters for active and non-active behavior traits have been estimated. Our heritability estimate for lying or sleeping is in agreement with the heritability for children sleeping duration [39]. A quite larger heritability was estimated for total daily sleep duration (daytime sleep duration plus nighttime sleep duration), considering 53 pairs of monozygotic and dizygotic female twins [40]. However, such an estimate might be biased due to a large proportion of common environmental effects in twins' samples.

The low heritability estimates for ET indicate partial genetic control of temperature regulation mechanisms but a stronger impact due to environmental effects and production levels [41]. Heritabilities for rectal temperature were larger in the range from 0.15 to 0.17 [41,42]. Nevertheless, regarding trait definition, there is a difference when measuring surface or rectal temperature [42]. Environmental temperature had a stronger impact on surface ET than on rectal and core body temperature [42,43]. Hence, heritabilities for rectal temperature were larger in the range from 0.15 to 0.17 [41,44]. The complex definition of welfare indices and the

inclusion of several antagonistic related traits might explain the quite small heritabilities and repeatabilities for WEL-IP and WEL-IC. In conclusion, we suggest the utilization of welfare indices as a novel management tool and not as a selection instrument to improve an animal's welfare status via breeding.

## Multi-breed genome-wide association study

To our knowledge, this is the first study considering dense sequences of longitudinal behavior measurements of dual-purpose cows from grazing systems across countries, combined with high-throughput genomic marker data. On the basis of a multi-breed GWAS, we gained new insights into the genetic control of dual-purpose cattle behavior under grazing conditions, and we located some interesting chromosomal segments. Nevertheless, for the detection of causal functional mutations in ongoing studies, it is imperative to use denser SNP data or even sequence data and a larger sample of genotyped cows [45,46]. Regarding the response traits, DRP reflected the daily behavior expression more accurately than one single MEAN value. In the statistical models for DRP, all important environmental (fixed) effects influencing bovine behavior [47] were considered. For the dependent variable MEAN, pre-correction of the data only accounted for the 'breed-farm' effect. Consequently, we suppose that the MEAN from an extended observation period does not fully reflect the genetic variation of bovine behavior due to confounding environmental effects. Additionally, when referring to the multi-breed GWAS, only one significant SNP was detected via the MEAN approach, but seven significant SNPs were discovered using DRP.

The identification of the *DGAT1* gene on BTA14 supported our a priori hypothesis that (despite the small sample size) the multi-breed GWAS is an appropriate approach to identify putative causative variants and candidate genes. Using an FDR of 20%, the number of identified significant genetic variants, including SNP and potential candidate genes, was larger compared to the stricter Bonferroni correction. However, the risk of detecting false positive SNPs also increased. The consideration of accumulated effects from a set of SNPs ±50 Kb of a gene (set-based association) was very powerful for detecting potential candidate genes, as suggested in previous studies [48]. Some of the discovered potential candidate genes are linked to behavior traits or diseases in cattle [49], humans [50], pigs [51] or mice [52].

**Rumination.** Based on the five significant SNPs with FDR $<$ 20%, we detected 13 potential candidate genes for RUM. Mutations of the identified potential candidate gene *RNF170* were associated with autosomal dominant sensory ataxia in humans [53]. The putative candidate gene *RPS6KB2* is involved in innate immune response mechanisms in indigenous and crossbred cattle [54]. In addition, the gene *RPS6KB2* was differentially expressed in Angus cattle selected for low and high residual feed intake [49] and in bovine tuberculosis-infected and control cattle [55]. Other findings suggest an association of *RPS6KB2* with embryonic development in cattle [56]. The *PTPRCAP* gene is an additional identified potential candidate gene that is associated with RUM behavior. In humans, *PTPRCAP* is involved in defense response mechanisms and is a key regulator of lymphocyte activation [50].

The putative candidate gene *CaBP4*, coding for a neuronal Ca2+-binding protein, was expressed in photoreceptors in mice and regulated synaptic terminals [52]. Haeseleer et al. [52] concluded that *CaBP4*−/− mice have behaviors similar to those in patients with incomplete congenital stationary night blindness. Generally, *CaBP4* is involved in the process of signal transduction [57] and visual perception [58].

The identified potential candidate gene *TMEM134* influences obesity and atherosclerosis in adults [59]. Furthermore, *TMEM134* is involved in the prototypical inflammatory nuclear factor-κB (*NF-κB*) signaling pathway [59]. The modulation of downstream *NF-κB* signaling is

the most important characteristic for innate immune programming in chronic inflammation [60]. The identified potential candidate gene *PITPNM1* is associated with retinal degeneration and hypopyon in humans and is involved in pathways of metabolism and glycerophospholipid biosynthesis [61].

**Feeding.** The potential candidate gene *LCN15* for FEED is involved in the transport of glucose and other sugars, bile salts and organic acids, metal ions and amine compounds as well as the transport of vitamins and nucleosides [61]. As a member of the lipocalin gene family, *LCN2* influences obesity and diabetes in humans [62]. Furthermore, *LCN15* physiologically interacted with high glucose levels in enterocytes [62]. Extended periods for FEED indicate an increase in feed intake [34], implying higher levels of sugars, fatty acids, amino acids, and vitamins. Hence, cows with different FEED levels might differ regarding specific expression profiles for the potential candidate gene (*LCN15*).

The potential candidate gene *SLC25A27* is part of a recently identified genetic network associated with economically important traits in Wagyu x Limousin crossbred cattle [63]. Additionally, *SLC25A27* contributes to long chain fatty acid uptake [63] and controls several diseases in humans, such as Alzheimer's disease [64], oxidative stress [65], and fasting [66]. The mitochondrial uncoupling protein 4 encoded by the *SLC25A27* gene is involved in thermoregulatory heat production and metabolism in the brain [67].

**Basic activity.** Only one potential candidate gene (*PPM1E*) influenced BACT behavior in dual-purpose cattle. Accordingly, the dephosphorylation gene (*PPM1E*) was associated with feeding behavior in Danish Duroc boars [51]. Do et al. [51] assumed that *PPM1E* is mediated by 5'AMP-activated protein kinase (*AMPK*), which plays a key role in controlling energy balances. The enzyme *AMPK* is involved in hypothalamic glucose and nutrient sensing. Hence, due to the identified impact of *PPM1E* on activity traits in dual-purpose cattle and due to the strong correlation between feeding and activity (S2 Table), behavior across species is based on the same genetic mechanisms.

## Associations among behavior traits and between behavior and productivity

The behavior traits RUM, FEED, BACT, HACT and NACT were interdependent, implying that the expression of basic behavior is involved in similar physiological processes. Additionally, from a practical perspective, some strong associations were expected. For example, an increase of feed intake (FEED) implies intensification of rumination time (RUM). Behavior-related feed intake had negative genetic impacts on BACT, enhanced BACT (HACT), and resting/sleeping (NACT) [68]. An increase in rumination and feeding contributes to improved milk production [69], but intensification of "production behavior" implies less time for BACT, HACT and NACT. "Normal" daily BACT behavior of dual-purpose cows was in balance with sleeping behavior (NACT). However, during estrus or parturition, cows express excessive walking, mounting and overall restlessness behavior (HACT), while the usual resting habits decrease [70] and body temperature increases [71,72]. Interestingly, welfare indices were also associated with ET.

Cows with 1% higher levels for RUM and FEED produced 0.10 kg and 0.12 kg more milk [68], respectively, along with increased somatic cell count. A simultaneous increase of SCS is due to the antagonistic relationship between MY and SCS [73]. High levels of daily BACT positively correlated with body condition loss, implying a reduction in MY [74]. The positive impact of BACT on NACT might explain the negative regression coefficient of NACT on MY. In general, daily bovine behaviors, including RUM, FEED, BACT, HACT, and NACT, do not have a significant impact on Fat% and Pro%. However, improved welfare indices were associated with higher values for Fat% and Pro%. Currently, in practical breeding schemes, Fat%

and Pro% are used as indicators to assess the cows' energy status [75]. Additionally, based on the results from the present study, Fat% and Pro% might be suitable indicator traits for cattle welfare.

## Materials and methods

### Animal ethics statement

Genotype data were provided from the national breeding organizations. Phenotypes reflect the standard trait pattern from official milk recording schemes. Behavior recording was non-invasive.

### Breeds and herd location

The five dual-purpose cattle breeds with phenotypic sensor behavior data were from Germany (DE_DSN), Poland (PL_BS, PL_DSN), Slovenia (Sl_BS, Sl_Si) and Switzerland (CH_OBV, CH_Si) (Table 4). In Germany and Poland, dual-purpose cows were kept in organic university research herds. The German research farm belongs to the federal state of Hesse in the center of Germany, and the farm in Poland is close to the Baltic Sea. In Slovenia, data recording considered three commercial grazing herds located in mountainous regions in the western part of the country, at 920 m to 970 m above sea level. In Switzerland, one original Braunvieh near Lucerne and one Simmental herd in the region around Basel were chosen for the across-country analyses. All farming conditions reflect pasture-based production systems, allowing grazing for at least 6 hours per day from May until November. Herd sizes ranged from 24 to 250 cows.

### Phenotypic data

**Sensor traits.** For the electronic recording of behavior traits, dual-purpose cows were equipped with sensors implemented in ear tags (Dutch CowManager system Agis Automatisering BV). The validation and testing phase of ear tag sensors under grazing conditions covered a period from 1 May 2016 until 31 June 2016 [68]. After one month of adaptation, ongoing analyses considered sensor data from July 2016 until March 2018 from 319 cows. Only cows with at least 30 consecutive days of sensor recording were included in the overall database. Once implemented in the cow's left ear, the sensor system uses a 3-dimensional accelerometer to identify behavior categories based on location coordinates. The behavior categories were RMU, FEED, NACT, BACT, and HACT. In addition, the sensor systems use a

**Table 4. Data structure for the cattle breeds included in multi-breed GWAS and genetic parameter estimations.**

| Country | Breed | No. of cows with sensor behavior data | No. of genotyped cows with sensor behavior data | No. of longitudinal sensor behavior records | No. of sensor behavior records per cow |
|---|---|---|---|---|---|
| DE | DE_DSN | 69 | 46 | 22,718 | 329.25 |
| PL | PL_BS | 49 | 28 | 17,332 | 353.71 |
| | PL_DSN | 66 | 51 | 24,386 | 369.49 |
| Sl | SI_Si | 17 | 14 | 2,973 | 174.88 |
| | SI_BS1 | 20 | 20 | 3,617 | 180.85 |
| | SI_BS2 | 8 | 8 | 1,633 | 204.13 |
| CH | CH_OBV | 45 | 36 | 11,944 | 265.42 |
| | CH_Si | 45 | 43 | 12,446 | 276.58 |

DE_DSN = black and white dual-purpose (Germany); PL_BS = Brown Swiss (Poland); PL_DSN = black and white dual-purpose (Poland); Sl_Si = Simmental (Slovenia); Sl_BS = Brown Swiss (Slovenia); CH_OBV = dual-purpose Original Braunvieh (Switzerland); CH_Si = Simmental (Switzerland).

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digital surface temperature monitor to measure the mean hourly ET. The system detects RUM based on the typical repetitive ear movement due to chewing and regurgitation. Feeding is related to food intake, expressed through masticatory movement. The activity parameters are subcategorized into BACT, HACT and NACT. The state of BACT describes any kind of moderate ear movement resulting from walking, head shaking or other movements, which cannot be associated with the specific repetitive ear movement during RUM or FEED. High activity is due to increased BACT, e.g., during estrus periods and including mounting behavior. No activity refers to minimal ear movements, while sleeping or resting. The hourly percentage of time spent for every behavior category is transmitted through a wireless connection to a router. Afterwards, the hourly percentages for behavior traits were transformed into daily time percentages. Whenever the sensor records a certain behavior, such as RUM, it does not assign this time to another behavior trait. Additionally, to evaluate the five sensor behavior categories, two welfare indices (WEL-IP and WEL-IC) were created following the welfare quality assessment protocol® [76] (Table 5). In this regard, sensor traits were assigned a score of 0, 1 or 2 according to physiological thresholds. WEL-IP was the sum of the scores from the different sensor traits. WEL-IC based on WEL-IP, but considering additional constraints as described in Table 6.

**Production traits.** Test-day records were from lactations 1 to 12 and considered the calving years from August 2015 until February 2018. Test-day MY, Fat%, Pro% and the log-transformed somatic cell count (SCS) were available for 329 cows from Germany, Poland and Switzerland. Descriptive statistics of the sensor traits, welfare indices and test-day traits are listed in Table 7.

## Genotypes

The five dual-purpose breeds, two additional breeds from Germany (DE_DN and DE_HF) and one from Slovenia (Sl_HF) were genotyped with the *Illumina Bovine 50K Bead chip* version *2*, with the *Illumina Bovine 50K Bead chip* version *3*, and with a customized bovine 50K SNP chip (*IDB V3*), according to the Illumina Infinium assay protocol (Illumina Inc., San Diego, CA, USA). Quality controls of the genotype data were conducted using PLINK software [77], defining a minor allele frequency of 0.01 and a deviation from Hardy–Weinberg equilibrium of $p < 0.00001$. All SNPs had a call rate larger than 85%, and SNPs located on sex chromosomes were excluded. Cows with a call rate lower than 80% for all loci were excluded. Whenever the genomic relation between two animals was larger than 0.95, one animal was excluded. Sporadic missing SNPs were imputed by the BEAGLE version 3.3.2 [78]. After SNP data editing and imputation, 35,826 SNPs from 615 cows were available (Table 8), and 246 genotyped cows had sensor records.

The data used in the present study is available as supplementary file S1 File.

**Table 5. Point assignment for welfare indices using the welfare quality assessment protocol® [76].**

| | Rumination | | | Feeding | | | Basic Active | | | High Active | | | Not Active | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Opt | Max | Min | Opt | Max | Min | Opt | Max | Min | Opt | Max | Min | Opt | Max |
| **Range, %/d** | < 29.2 | 29.2–41.7 | > 41.7 | < 12.5 | 12.5–20.8 | > 20.8 | < 8.3 | 8.3–12.5 | > 12.5 | < 8.3 | 8.3–12.5 | > 12.5 | < 16.7 | 16.7–41.7 | > 41.7 |
| **Range, h/d** | < 7 | 7–10 | > 10 | < 3 | 3–5 | > 5 | < 2 | 2–3 | > 3 | < 2 | 2–3 | > 3 | < 4 | 4–10 | > 10 |
| **Points** | 0 | 2 | 1 | 0 | 2 | 1 | 0 | 2 | 1 | 1 | 2 | 0 | 1 | 2 | 0 |
| **Meaning** | Al | Norm | OK | Al | Norm | OK | Al | Norm | i.h. | - | Norm | i.h. | Al | Norm | Al |

Opt = optimum (normal) behavior range; Al = alarming (check animal or management); Norm = normal; OK = harmless, but not as good as Norm; i.h. = possibly in heat; the welfare index point of every observation can be calculated by summing the points for rumination, feeding, basic active, high active, and not active.

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**Table 6. Composed welfare index classes based on the welfare quality assessment protocol® [76].**

| Welfare index classes | Meaning | Points[a] | Criteria |
|---|---|---|---|
| **1** | Excellent | > 6 (7–10) | 1) at least 1 point in every sensor trait category; 2) rumination and feeding should have 2 points according to Table 5. |
| **2** | Acceptable | 5–9 | |
| **3** | Poor (health/welfare impairment) | < 5 | |

[a] Sum of welfare points across rumination, feeding, basic active, high active, and not active for each observation according to Table 5.

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## Population structure and breed assignment

PCA was conducted to account for potential population stratification prior to GWAS and to explore the genetic diversity of the European cow dataset. PCA based on the genomic relationship matrix was generated in GCTA [79]. In a second step, a breed assignment analysis was conducted using the WIDDE program [16]. The WIDDE cattle database contained over 750,000 SNPs from 3,951 individuals, which belong to 129 different populations [16]. The broad variety of local cattle populations in WIDDE represents the bovine genetic diversity and covers the three main cattle groups, i.e., European and African taurine (*Bos taurus*) as well as zebus (*Bos indicus*) [16]. The allele proximity between the genotyped populations from this study and the populations represented in the world reference dataset in WIDDE were estimated using supervised clustering [16]. A convergence criterion of 0.01 for log-likelihood values was defined when calculating the percentage of ancestry proportions between each genotyped cow and the 129 populations from the WIDDE world reference dataset.

## Genetic parameter estimations

For the estimation of genetic parameters, genomic and pedigree relationship matrices were combined. In additional analyses, only the pedigree relationship matrix was considered. The pedigree consisted of 8,798 animals and was traced back as far as possible. The oldest ancestors in the pedigree were born in 1944 for Germany, in 1981 for Poland, in 1990 for Slovenia, and

**Table 7. Descriptive statistics for sensor behavior and production traits.**

| Trait | No. of observations | No. of cows | Mean | SD | Min. | Max. |
|---|---|---|---|---|---|---|
| **RUM** | 97,049 | 319 | 34.13 | 7.07 | 5.94 | 81.36 |
| **FEED** | 97,049 | 319 | 23.87 | 8.47 | 0.19 | 66.32 |
| **BACT** | 97,049 | 319 | 8.45 | 5.28 | 0.16 | 50.75 |
| **HACT** | 97,049 | 319 | 7.76 | 3.22 | 0.18 | 33.78 |
| **NACT** | 97,049 | 319 | 25.79 | 7.51 | 4.58 | 72.83 |
| **ET** | 97,049 | 319 | 24.66 | 4.59 | 2.23 | 38.28 |
| **WEL-IP** | 97,049 | 319 | 6.27 | 1.49 | 0 | 10 |
| **WEL-IC** | 97,049 | 319 | 2.04 | 0.42 | 1 | 3 |
| **MY** | 6,571 | 329 | 19.33 | 6.3 | 1.6 | 47.2 |
| **Fat%** | 6,546 | 329 | 4.1 | 0.67 | 1.84 | 7.98 |
| **Pro%** | 6,546 | 329 | 3.43 | 0.41 | 2.12 | 5.5 |
| **SCS** | 6,546 | 329 | 2.43 | 1.54 | -1.32 | 10.5 |

RUM = rumination; FEED = feeding; BACT = basic active; HACT = high active; NACT = not active; ET = ear temperature; WEL-IP = welfare index point; WEL-IC = welfare index class; MY = milk yield (in kg); Fat% = fat percentage; Pro% = protein percentage; SCS = somatic cell score.

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**Table 8. Genotype data of five cattle breeds included in PCA, WIDDE and multi-breed GWAS.**

| Country | Breed | No. of cows | No. of cows after SNP quality control |
|---|---|---|---|
| DE | DE_DSN | 266 | 266 |
| | DE_DN | 20 | 20 |
| | DE_HF | 50 | 50 |
| PL | PL_BS | 34 | 34 |
| | PL_DSN | 59 | 59 |
| Sl | Sl_Si | 46 | 44 |
| | Sl_BS | 36 | 36 |
| | Sl_HF | 14 | 14 |
| CH | CH_OBV | 48 | 46 |
| | CH_Si | 48 | 46 |

DE_DSN = black and white dual-purpose (Germany); DE_DN = red and white dual-purpose (Germany); DE_HF = Holstein Friesian (Germany); PL_BS = Brown Swiss (Poland); PL_DSN = black and white dual-purpose (Poland); Sl_Si = Simmental (Slovenia); Sl_BS = Brown Swiss (Slovenia); Sl_HF = Holstein Friesian (Slovenia); CH_OBV = dual-purpose Original Braunvieh (Switzerland); CH_Si = Simmental (Switzerland).

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in 1917 for Switzerland. Variance components of sensor traits were estimated via univariate animal models using the AIREML procedure, as implemented in the DMU software package [80]. The statistical model (1) in matrix notation was defined as follows:

$$\mathbf{y} = \mathbf{Xb} + \boldsymbol{Z_1 a} + \boldsymbol{Z_2 p} + \boldsymbol{e} \quad (1)$$

where **y** was the observation vector for sensor traits and indices (RUM, FEED, NACT, BACT, HACT, ET, WEL-IP and WEL-IC); **b** was the vector of fixed effects including the combined breed-farm effect, the year-month effect for the measuring date, and the age of cows as a fixed linear regression; **a** was the vector for additive genetic effects; **p** was the vector for permanent environmental effects for the cows with repeated measurements; **e** was the vector of random residual effects, and **X**, $\mathbf{Z_1}$, and $\mathbf{Z_2}$ were incidence matrices for **b**, **a**, and **p**, respectively. The assumed variance-covariance structure was $\mathbf{a} \sim N(0, K\sigma_a^2)$, where $\sigma_u^2$ was the genetic variance, **K** was the **A** matrix, or the combined **H** matrix when blending **A** and the weighted genomic relationship matrix ($\mathbf{G_w}$) [81]. $\mathbf{G_w}$ was calculated as follows:

$$\mathbf{G_w} = (0.95 \times \mathbf{G} + 0.05 \times \mathbf{A_{22}})$$

where $\mathbf{A_{22}}$ is the submatrix of the pedigree-based relationship matrix for genotyped animals. Estimated breeding values (EBV) from model 1 and consideration of the **A** matrix were the databases for the calculation of DRP according to Garrick et al. [82]. Only animals with a DRP weight larger than 0.2 were considered for the ongoing GWAS [83].

The genetic-statistical model (2) used for test-day production traits and SCS based on the **A** matrix was defined as follows:

$$\mathbf{y} = \mathbf{Xb} + \boldsymbol{Z_1 a} + \boldsymbol{Z_2 p} + \boldsymbol{e} \quad (2)$$

where **y** was the observation vector for MY, Fat%, Pro%, and SCS; **b** was the vector of fixed effects including the breed-farm and calving-year-season effects, and the lactation curve modeled via Legendre polynomials of order three for days in milk; **a** was the vector for additive genetic effects based on the **A** matrix; **p** was the vector for permanent environmental effects for the cows with repeated measurements; **e** was the vector of random residual effects, and **X**,

$\mathbf{Z_1}$, and $\mathbf{Z_2}$ were the incidence matrices for **b**, **a**, and **p**, respectively. Again, EBV were de-regressed to obtain DRP for test-day production traits and SCS.

## Multi-breed GWAS

Single-trait multi-breed GWAS was performed using the software package GCTA [79]. In this regard, we considered the leave-one-chromosome-out (*loco*) option. Dependent variables (i.e., our phenotypes) were MEAN and DRP. For testing single-locus SNP effects, the following statistical model (3) was used:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Wg} + \mathbf{Zu} + \mathbf{e} \quad (3)$$

where **y** was the vector of DRP or MEAN for RUM, FEED, NACT, BACT, HACT, ET, WEL-IP, and WEL-IC, as well as DRP for production traits; **b** was a vector of fixed effects considering only the overall mean for DRP and additionally the breed-farm effect for MEAN; **g** was the vector for additive fixed effects of the candidate SNP; **u** was the vector for polygenic effects considering all SNPs but excluding SNPs from the chromosome where the candidate SNP was located; and **e** was the vector of random residual effects; **X**, **W**, and **Z** were incidence matrices for **b**, **g**, and **u**, respectively. The Bonferroni threshold for SNP associations was $p_{\mathrm{Bonf}}$ = 0.05/(number of SNP) = 0.05/35,826 = 4.47 x $10^{-7}$. The FDR as introduced by Benjamini and Hochberg [84] was a further significance threshold for genome-wide associations. The FDR to detect candidate SNPs for behavior traits and test-day production traits was set to 20%.

## Candidate gene annotation

The associated potential candidate genes were identified via a gene-based test in GCTA and applying the *fastBAT* option [48]. The database (version UMD3.1), including gene locations and start and end positions for the bovine genes, was downloaded from Ensembl [50]. A total of 24,616 gene ID entries were originally available in the database, but only 17,545 genes on chromosomes 1 to 29 were included in further analyses (i.e., exclusion of pseudogenes according to [76,85,86]). In the first step, all SNPs from the GWAS were mapped to the genes, considering a window of 50 kb upstream and 50 kb downstream from the genes. Subsequently, *P-values* considering the set of SNPs within the window were used simultaneously for candidate gene detection. The *P-values* of genes were adjusted according to FDR (significance threshold $< 20\%$). In the last step, physiological functions and positions of candidate genes were inferred based on information from the Ensembl [50], NCBI [87], UniProt [88] and Genecard [61] databases.

## Causal associations

In epidemiology, Mendelian randomization uses genetic variants as instrumental variables to test for the causal inference between an exposure and an outcome [89]. Hence, we assume an instrumental variable z, representing the SNP genotype. The exposure x considered one of the behavior traits, and the outcome y was the test-day productivity or SCS. Assuming uncorrelated $z$ and uncorrelated residuals when regressing $y$ on $x$ and $cov(z, x) \neq 0$, the regression coefficient of $\hat{b}_{yx}$ was [90]:

$$\hat{b}_{yx} = \frac{cov(z,\ y)}{cov(z,\ x)} = \frac{\frac{cov(z,y)}{var(z)}}{\frac{cov(z,x)}{var(z)}} = \frac{\hat{b}_{yz}}{\hat{b}_{xz}} \quad (4)$$

where $\hat{b}_{yz}$ and $\hat{b}_{xz}$ were the estimated SNP effects from GWAS when using $y$ and $x$ as

phenotypes, respectively. The variance of $\hat{b}_{yx}$ was:

$$var(\hat{b}_{yx}) = \frac{var(e_{yx})}{n \cdot var(x) \cdot R^2_{xz}} = \frac{var(y) \cdot (1 - \rho^2_{xy})}{n \cdot var(x) \cdot \frac{2 \cdot p \cdot (1-p) \cdot \hat{b}^2_{xz}}{var(x)}} = \frac{var(y) \cdot (1 - \rho^2_{xy})}{n \cdot 2 \cdot p \cdot (1-p) \cdot \hat{b}^2_{xz}} \quad (5)$$

where $var(e_{xy})$ was the residual variance when fitting $x$ as a fixed regression to explain $y$; n was the sample size; $var(x)$ was variance of trait $x$; and $R^2_{xz}$ was the proportion of variance $x$ explained by $z$, which equaled $\frac{2 \cdot p \cdot (1-p) \cdot \hat{b}^2_{xz}}{var(x)}$. In Eq (5), $var(y)$ was the variance of trait $y$; $\rho^2_{xy}$ was the squared correlation between trait $x$ and trait $y$; $p$ was the allele frequency, and $\hat{b}^2_{xz}$ was the squared SNP effect estimate from GWAS for trait $x$. The test statistic $\frac{T_{MR} = \hat{b}^2_{yx}}{var(\hat{b}_{yx})}$ followed $\chi^2_1$ [91], which was used to test the significance of $\hat{b}_{yx}$.

To fulfill the precondition of $cov(z, x) \neq 0$, 445 homologous genes in the human and bovine genome that were involved in the biological process of behavior were searched and downloaded from AmiGO2, a Gene Ontology database [85,86]. A total of 1,011 SNPs within a window of 50 kb up- and downstream of the 445 homologous genes were considered.

Afterwards, the GWAS estimates for the 1,011 SNP were transmitted into a self-modified version of the GSMR package [92] in R to calculate the variance of $\hat{b}_{yx}$ for a small sample size. The aforementioned SNP was filtered according to the following criteria: 1) *P-value* of the SNP lower than 0.05 to meet the assumption of $cov(z, x) \neq 0$; and 2) LD between SNP lower than 0.25 to prune highly correlated SNPs. After filtering, the number of SNPs for behavior traits and welfare indices varied between 36 (for BACT) and 64 (for RUM).

## Supporting information

**S1 Fig. Manhattan plot and Q-Q plot from GWAS based on the mean (MEAN) and de-regressed proofs (DRP) of daily not active time.** The red line is the significance threshold line for the Bonferroni correction of 5%, and the green dots represent significant SNP according to the false discovery rate of 20%.
(PDF)

**S2 Fig. Manhattan plot and Q-Q plot from GWAS based on de-regressed proof of daily rumination time.** The red line is the significance threshold line for the Bonferroni correction of 5%, and the green dots represent significant SNP according to the false discovery rate of 20%.
(PDF)

**S3 Fig. Manhattan plot and Q-Q plot from GWAS based on de-regressed proof of daily feeding time.** The red line is the significance threshold line for the Bonferroni correction of 5%, and the green dots represent significant SNP according to the false discovery rate of 20%.
(PDF)

**S4 Fig. Manhattan plot and Q-Q plot from GWAS based on de-regressed proof of test-day fat percentage.** The red line is the significance threshold line for the Bonferroni correction of 5%, and the green dots represent significant SNP according to the false discovery rate of 20%.
(PDF)

**S1 Table. Potential candidate genes associated with animal behavior traits.**
RUM = rumination; FEED = feeding; BACT = basic active; BTA = Bos taurus chromosome; DRP = de-regressed proof; MEAN = mean of observations; functions derived from Ensembl[1],

NCBI[2], UNIPROT[3], and GeneCard[4].
(DOCX)

**S2 Table. Genetic (above diagonal) and phenotypic (below diagonal) correlations among sensor behavior.** Correlations estimated from bivariate models with the same fixed and random effects as model (1) and standard errors in brackets. RUM = rumination; FEED = feeding; BACT = basic active; HACT = high active; NACT = not active; ET = ear temperature; WEL-IP = welfare index point; WEL-IC = welfare index class; nc = did not converge.
(DOCX)

**S1 File. Raw phenotypes, genotypes, pedigrees, and values to build Figs 1 and 2 are available in the compressed file.**
(ZIP)

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## Author Contributions

**Conceptualization:** Sven König.

**Data curation:** Carsten Scheper, Tomasz Sakowski, Marija Klopčič, Beat Bapst.

**Formal analysis:** Tong Yin, Maria Jaeger, Carsten Scheper.

**Funding acquisition:** Sven König.

**Investigation:** Gregorz Grodkowski.

**Software:** Gregorz Grodkowski.

**Supervision:** Sven König.

**Validation:** Sven König.

**Writing – original draft:** Tong Yin, Maria Jaeger.

**Writing – review & editing:** Tomasz Sakowski, Beat Bapst, Sven König.

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## 2.8. Original research paper 8

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# Heritabilities and genetic correlations in the same traits across different strata of herds created according to continuous genomic, genetic, and phenotypic descriptors

**Tong Yin and Sven König**[1]
Institute of Animal Breeding and Genetics, Justus-Liebig-University of Gießen, 35390 Gießen, Germany

## ABSTRACT

The most common approach in dairy cattle to prove genotype by environment interactions is a multiple-trait model application, and considering the same traits in different environments as different traits. We enhanced such concepts by defining continuous phenotypic, genetic, and genomic herd descriptors, and applying random regression sire models. Traits of interest were test-day traits for milk yield, fat percentage, protein percentage, and somatic cell score, considering 267,393 records from 32,707 first-lactation Holstein cows. Cows were born in the years 2010 to 2013, and kept in 52 large-scale herds from 2 federal states of north-east Germany. The average number of genotyped cows per herd (45,613 single nucleotide polymorphism markers per cow) was 133.5 (range: 45 to 415 genotyped cows). Genomic herd descriptors were (1) the level of linkage disequilibrium ($r^2$) within specific chromosome segments, and (2) the average allele frequency for single nucleotide polymorphisms in close distance to a functional mutation. Genetic herd descriptors were the (1) intra-herd inbreeding coefficient, and (2) the percentage of daughters from foreign sires. Phenotypic herd descriptors were (1) herd size, and (2) the herd mean for nonreturn rate. Most correlations among herd descriptors were close to 0, indicating independence of genomic, genetic, and phenotypic characteristics. Heritabilities for milk yield increased with increasing intra-herd linkage disequilibrium, inbreeding, and herd size. Genetic correlations in same traits between adjacent levels of herd descriptors were close to 1, but declined for descriptor levels in greater distance. Genetic correlation declines were more obvious for somatic cell score, compared with test-day traits with larger heritabilities (fat percentage and protein percentage). Also, for milk yield, alterations of herd descriptor levels had an obvious effect on heritabilities and genetic correlations. By trend, multiple trait model results (based on created discrete herd classes) confirmed the random regression estimates. Identified alterations of breeding values in dependency of herd descriptors suggest utilization of specific sires for specific herd structures, offering new possibilities to improve sire selection strategies. Regarding genomic selection designs and genetic gain transfer into commercial herds, cow herds for the utilization in cow training sets should reflect the genomic, genetic, and phenotypic pattern of the broad population.
**Key words:** genotype × environment interactions, test-day production trait, genetic and genomic herd descriptors, random regression model

## INTRODUCTION

The existence of genotype by environment interactions (**G×E**) indicates that different genotypes show different trait reactions in different environments. Obvious G×E imply re-rankings of genotypes in different environments. The physiological background might be that different genes are switched on and off with environmental changes. Such genomic determinants of G×E were also studied via gene expressions (Grishkevich and Yanai, 2013).

The general quantitative-genetic proof for the detection of G×E is based on Falconer's concept (Falconer and Mackay, 1996), that is, defining same traits in different environments as different traits, and applying multiple trait models. As an indicator for a possible G×E, Robertson (1959) recommended a genetic correlation threshold of 0.80. During the past decades, a multiplicity of G×E studies applied the classical multiple trait approach (König et al., 2005). Genetic correlations substantially lower than 0.80 were identified for pronounced differences of production systems within countries [e.g., "grazing" versus "conventional" (Boettcher et al., 2003)], or for extremely diverse countries [e.g., "Kenya" versus "United Kingdom" (Ojango and Pollott, 2002)]. König et al. (2002) stretched this concept by stratifying data according to production

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[1]Corresponding author: sven.koenig@agrar.uni-giessen.de

systems plus considering cow sire characteristics. Cow sire characteristics addressed the sire origin (i.e., the creation of specific data sets only including daughters from foreign proven sires, or proven sires from Germany, or national test bulls). Production systems were defined as family farms from the western (west) versus large-scale farms from the eastern (east) part of Germany. Genetic correlations for protein yield defined in the east and west as different traits were close to 1 for the cow data set from proven sires, but substantially decreased when exclusively considering daughters from test bulls. König et al. (2002) assumed the effect of the size of the data sets (substantially lower number of cows from test bulls), and of genetic connectedness between the data sets, on genetic correlation estimates. Recently, they continued their previous study by creating identical data sets for the different cow sire groups. In this regard, "identical datasets" implied an identical number of cow records, and an equal number and distribution of daughter records per sire category (Table 1). However, the general trend still existed: genetic correlations decreased for an increased herd size in the east region, and the effect was more pronounced in test bull daughters compared with daughters from proven sires (Table 1). One possible explanation might be the effect of selection strategies. In a theoretical approach, Via and Lande (1985) studied G×E and additionally considered the type of selection on phenotypic plasticity. Selection itself influenced genetic architectures within populations (Bastiaansen et al., 2012).

Results from G×E studies (Table 1) were the motivation for ongoing research in this regard (i.e., to identify possible effects of genetic or genomic herd or sub-population characteristics on genetic correlations for the same traits recorded in different environments). In the genomic era, the availability of high-throughput genomic SNP marker data from commercial cows allows detailed insight into specific chromosomal regions and genetic architectures. In the German "cow calibration group" project for Holstein cows (Yin and König, 2016), more than 20,000 cows from large-scale herds have been genotyped on a 50K SNP platform. For this data structure, Naderi et al. (2016) identified the effect of disease incidences in cow training sets on accuracies of genomic predictions. In consequence, they suggested a training set composition reflecting disease incidences in the broad population.

Random regression or reaction norm methodology was applied to study the effect of continuous environmental herd descriptors or phenotypic herd means on genetic (co)variance components. For example, G×E for production and functional traits were identified along gradients for within-herd temperature-humidity indices (Brügemann et al., 2011, 2013), or when considering mean values for the intra-herd BCS (Calus and Veerkamp, 2003). In the genomic era, random regression models or reaction norm models were enhanced by modeling genomic instead of pedigree relationships (Yin et al., 2014), by incorporating one-step methodology (Tsuruta et al., 2015), and by estimating intercept and slope of the regression line using SNP marker data (Streit et al., 2013; Nguyen et al., 2016). So far, availability of genomic marker data on an animal level enhanced statistical modeling. Furthermore, on a herd level, genetic and genomic characteristics of herds included in cow training sets might influence (1) genetic (co)variance components between the 2 strata of data (training set and remaining population), and in conse-

**Table 1**. Structure of the data and genetic correlations for protein yield between strata of the data for 305-d lactation protein yield[1]

| Region | | Sire category | No. of cows | | No. of sires in common | Average no. of daughters of sires in common | | $r_g$ | Reference[2] |
|---|---|---|---|---|---|---|---|---|---|
| A | B | | Region A | Region B | | Region A | Region B | | |
| West | East | All | 43,926 | 94,335 | 586 | 51 | 91 | 0.93 | A |
| West | East | Proven | 26,149 | 77,414 | 247 | 81 | 169 | 0.96 | B |
| West | East | Test bull | 9,054 | 11,556 | 119 | 42 | 2 | 0.88 | B |
| West | East$^{>150}$ | All | 43,926 | 34,368 | 291 | 68 | 65 | 0.87 | A |
| West | East$^{>150}$ | Proven | 26,149 | 27,999 | 161 | 51 | 97 | 0.89 | B |
| West | East$^{>150}$ | Test bull | 9,054 | 4,906 | 105 | 18 | 2 | 0.79 | B |
| West | East | All | 7,013 | 7,004 | 100 | 31 | 12 | 0.91 | C |
| West | East | Proven | 7,000 | 6,996 | 100 | 31 | 12 | 0.95 | C |
| West | East | Test bull | 7,011 | 7,003 | 100 | 31 | 12 | 0.84 | C |
| West | East$^{>150}$ | All | 4,500 | 4,497 | 291 | 13 | 6 | 0.84 | C |
| West | East$^{>150}$ | Proven | 4,499 | 4,506 | 161 | 13 | 6 | 0.88 | C |
| West | East$^{>150}$ | Test bull | 4,502 | 4,501 | 85 | 13 | 6 | 0.74 | C |

[1]West and east denote regions (states) from western and eastern Germany; East$^{>150}$ denotes large-scale herds with at least 150 cows from first lactation per herd-calving year.

[2]A: König et al. (2005); B: König et al. (2002); C: König (2017).

quence, (2) genomic prediction accuracies. The level of relationships between training and testing sets was one important parameter influencing genomic prediction accuracies (e.g., Habier et al., 2010; Clark et al., 2012; Pszczola et al., 2012). However, differences in prediction accuracies and genetic parameter estimates were observed when either using pedigree- or genomic-based relationship matrices (Veerkamp et al., 2011).

In consequence, in the present study, we used phenotypes and high-throughput genotypes (50K SNP chip panel) from cows kept in large-scale contract herds to create herd descriptors on phenotypic (herd size and nonreturn rate), genetic (inbreeding and daughter percentage from foreign sires), and genomic [level of linkage disequilibrium (**LD**) and allele frequencies] scales. The aims of this study were (1) to estimate genetic parameters in dependency of herd descriptors, and (2) to estimate genetic correlations in same traits across herd descriptor levels. Results indicate the effect of herd characteristics on quantitative-genetic (co)variance components and breeding values in the context of possible G×E, with practical relevance when selecting cow herds for their use in cow calibration groups.

## MATERIALS AND METHODS

### Cow Traits

The test-day data set consisted of 267,393 records from 32,707 first lactation Holstein cows, born in 2010 to 2013. Test-day traits included milk yield (**MY**), fat percentage (**fat%**), protein percentage (**Pro%**), and SCS. For all test-day traits, cows had at least 3 repeated measurements. Descriptive statistics for the test-day traits are listed in Table 2. Cows were from 52 large-scale herds, located within 2 federal states of north-east Germany. Herd size ranged from 246 to 2,067 cows, with an average of 654.14 cows per herd. The average number of records per contemporary group (herd-test-day) was 139.41. The 32,707 cows were daughters from 681 sires, indicating an average of 48.03 daughters per sire. The daughter distribution within sires was as follows: 150 sires had 2 to 10 daughters, 332 sires had 11 to 40 daughters, 149 sires had 41 to 100 daughters, and 50 sires had more than 100 daughters. The most influential sire had 938 daughters. Each sire had daughter records in at least 2 different herds.

### Herd Descriptors

***Genomic Herd Descriptors.*** Genotyping was performed with the Illumina Bovine 50K SNP BeadChip V2 (applied to 4,569 cows; Illumina, San Diego, CA), and with the Illumina Bovine Eurogenomics 10K low density chip (applied to 2,047 cows). The low density genotypes were imputed to the 50K panel as used in the national official German genomic evaluations, resulting in 45,613 SNP for all animals. The average number of genotyped cows per herd was 133.5, ranging from 45 to 415 genotyped cows.

***LD.*** The level of LD between all possible SNP pairs within 25 kb windows was calculated based on pairwise $r^2$ (Hill and Robertson, 1968), and applying the PLINK software package (Purcell et al., 2007). Intra-herd $r^2$ was the average $r^2$ from all SNP pairs, considering (1) the genotyped cows in the respective herd, and (2) SNP with a minor allele frequency of 0.05 within the 25-kb window. First, we focused on 397 SNP within the 40- to 60-Mbp segment on chromosome 6 ($\mathbf{r_{chr6}^2}$), because in a meta-analysis (Khatkar et al., 2004), QTL in this region contributed with significant effects on milk yield, fat yield, protein yield, fat percentage, and protein percentage. Second, we calculated intra-herd $r^2$ based on 786 SNP within the 0 to 40 Mbp segment on chromosome 14 ($\mathbf{r_{chr14}^2}$), to consider LD in the *DGAT1* region (Thaller et al., 2003).

***Allele Frequency.*** We calculated the intra-herd allele frequency ($\boldsymbol{p}$) for one specific SNP (*ARS-BFGL-NGS-4939*) located on chromosome 14. The exact position for this SNP is at 1,801,116 bp in the *DGAT1* gene (Jiang et al., 2010; Minozzi et al., 2013). The *DGAT1* gene start and end positions are 1,795,351 and 1,804,562 bp on chromosome 14. The *DGAT1 K232A* polymorphism explained 50% of the genetic variance for fat percentage (Schennink et al., 2007). Accordingly, there is selection pressure on the *DGAT1* gene, with different intensity and with different direction in different herds. Selection itself has a strong effect on the *ARS-BFGL-NGS-4939* allele frequency. Allele frequency changes might influence selection response.

**Table 2**. Descriptive statistics for test-day traits

| Trait | No. of records | No. of cows | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|---|
| Milk yield (kg) | 267,393 | 32,707 | 29.71 | 6.35 | 2.00 | 67.20 |
| Fat percentage (%) | 267,393 | 32,707 | 3.89 | 0.67 | 1.60 | 10.31 |
| Protein percentage (%) | 267,393 | 32,707 | 3.36 | 0.33 | 2.03 | 6.86 |
| SCS | 266,849 | 32,620 | 2.38 | 1.59 | −3.64 | 9.64 |

**Table 3**. Descriptive statistics for the continuous herd descriptors

| Herd descriptor | Mean | SD | CV | Minimum | Maximum |
|---|---|---|---|---|---|
| $r^2$ on chromosome 6 between 40 and 60 Mbp ($r_{chr6}^2$) | 0.28 | 0.01 | 0.04 | 0.25 | 0.32 |
| $r^2$ on chromosome 14 between 0 and 40 Mbp ($r_{chr14}^2$) | 0.31 | 0.01 | 0.03 | 0.29 | 0.36 |
| Allele frequency of the SNP located in the *DGAT1* gene ($p$) | 0.69 | 0.05 | 0.07 | 0.61 | 0.82 |
| Pedigree-based inbreeding coefficient ($F$) | 0.05 | 0.003 | 0.06 | 0.04 | 0.05 |
| Percentage of cows with a non-European Union sire (sire%) | 0.10 | 0.08 | 0.80 | 0.00 | 0.39 |
| Herd size (HS) | 654.14 | 391.51 | 0.60 | 246 | 2,067 |
| Nonreturn rate after 56 d (NRR56) | 0.50 | 0.07 | 0.14 | 0.35 | 0.68 |

### Genetic Herd Descriptors

***Inbreeding Coefficient.*** Intra-herd pedigree-based inbreeding coefficients (***F***) were calculated using the software package CFC (Sargolzaei et al., 2006). We considered all of the 1,292,113 animals in the pedigree file when calculating the inbreeding coefficients of the 32,707 active cows.

***Percentage of Cows with a Non-European Union Sire.*** We calculated the percentage of cows with a non-European Union (**EU**) sire in each herd (**sire%**) based on sire origin information from national genetic evaluation databases. Non-EU sires mostly included Holstein sires from the United States and Canada (95% of the non-EU sires). Among the 681 sires, 77 non-EU sires were from the United States, 15 non-EU sires were from Canada, and 1 non-EU sire was from Australia. The remaining sires were from European countries, especially Germany.

### Phenotypic Herd Descriptors

***Herd Size.*** We considered cows from calving years 2010 to 2013 for the calculation of intra-herd herd sizes (**HS**).

***Nonreturn Rate After 56 d.*** We characterized herds on a continuous phenotypic "cow trait scale" via herd means for nonreturn rates after 56 d (**NRR56**) from the calving years 2010 to 2013. Nonreturn rate as a phenotypic descriptor reflects the effect of natural selection, which occurred in female fertility traits rather than in production traits (Bishop, 1964).

Descriptive statistics for all herd descriptors are given in Table 3. To prepare data for the multiple trait approach, we grouped herds into distinct classes according to their herd descriptor means. For all herd descriptors ($r_{chr6}^2$, $r_{chr14}^2$, $p$, $F$, sire%, HS, and NRR56), we created 3 groups, defined as small, middle, and high. We ordered herds according to herd descriptors, and we allocated an equal number of herds to small, middle, and high; the small group included the first 17 herds with the smallest value for the respective descriptor, middle represented herds from rank 18 to rank 33, and the remaining 17 herds with the largest means were considered in the high herd group. In a random herd allocation scenario (random), herds randomly received consecutive numbers from 1 to 52. According to the assigned random numbers, herds were allocated to small, middle, or high. We used 50 replicates for the random herd number allocations. Hence, presented genetic parameters were averages from the 50 replicates, along with corresponding standard deviation.

### Statistical Models

Two different statistical models based on the AI-REML algorithm as implemented in the DMU package (Madsen and Jensen, 2013). The first model was a random regression sire model (**RRM**), using the herd means for $r_{chr6}^2$, $r_{chr14}^2$, $p$, $F$, sire%, HS, and NRR56 as continuous descriptors. In matrix notation, the RRM was defined as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Wp} + \mathbf{e},$$

where $\mathbf{y}$ was a vector of records for test-day traits; $\mathbf{b}$ was a vector of fixed effects including herd-test-day, fixed regressions on age at first calving (in month) modeled with Legendre polynomials of order 3, and fixed regressions on DIM also modeled with Legendre polynomials of order 3; $\mathbf{u}$ was a vector of random regression coefficients for additive-genetic sire effects modeled with linear regressions for the continuous herd descriptors (consecutive runs for the different herd descriptors); $\mathbf{p}$ was a vector of permanent environmental effects for the cows; and $\mathbf{e}$ was a vector of random residual effects. $\mathbf{X}$, $\mathbf{Z}$, and $\mathbf{W}$ were incidence matrices for $\mathbf{b}$, $\mathbf{u}$, and $\mathbf{p}$, respectively. Random effects were assumed to follow a normal distribution with zero means. The variance-covariance structure for random effects was

$$\text{var}\begin{bmatrix}\mathbf{u}\\ \mathbf{p}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{G}\otimes\mathbf{A_u} & 0 & 0\\ 0 & \boldsymbol{\sigma}_\mathbf{p}^2\mathbf{I_p} & 0\\ 0 & 0 & \boldsymbol{\sigma}_\mathbf{e}^2\mathbf{I_n}\end{bmatrix},$$

where $\mathbf{G}$ was a 2 × 2 (co)variance matrix of random regression coefficients for the additive genetic effect; $\mathbf{A_u}$ was an additive genetic relationship matrix for sires; $\boldsymbol{\sigma}^2_{\mathbf{p}}$ and $\boldsymbol{\sigma}^2_{\mathbf{e}}$ were variances for the permanent environmental and residual effect, respectively; $\mathbf{I_p}$ and $\mathbf{I_n}$ were identity matrices for $p$ cows and $n$ observations, respectively; and ⊗ denotes the Kronecker product.

Second, we applied multiple trait repeatability sire models (**MTRM**). Following the concept by Falconer and Mackay (1996), we defined same traits recorded in different herd descriptor groups as different traits. Accordingly, we assumed nonexisting phenotypic and residual covariances among traits, because the same cow only represented one herd descriptor class.

In matrix notation, the MTRM including the 3 traits was

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\\ \mathbf{y}_3\end{bmatrix} = \begin{bmatrix}\mathbf{X}_1\mathbf{b}_1 + \mathbf{Z}_1\mathbf{u}_1 + \mathbf{W}_1\mathbf{p}_1 + \mathbf{e}_1\\ \mathbf{X}_2\mathbf{b}_2 + \mathbf{Z}_2\mathbf{u}_2 + \mathbf{W}_2\mathbf{p}_2 + \mathbf{e}_2\\ \mathbf{X}_3\mathbf{b}_3 + \mathbf{Z}_3\mathbf{u}_3 + \mathbf{W}_3\mathbf{p}_3 + \mathbf{e}_3\end{bmatrix},$$

where $\mathbf{y_1}$, $\mathbf{y_2}$, and $\mathbf{y_3}$ were vectors of test-day records for small, middle, and high, respectively; $\mathbf{b_1}$, $\mathbf{b_2}$, and $\mathbf{b_3}$ were vectors of fixed effects for the 3 traits including the same fixed effects as specified in the RRM (apart from the continuous herd descriptor); $\mathbf{u_1}$, $\mathbf{u_2}$, and $\mathbf{u_3}$ were vectors of additive-genetic sire effects for the 3 traits; $\mathbf{p_1}$, $\mathbf{p_2}$, and $\mathbf{p_3}$ were vectors of permanent environmental effects for the cows in the 3 data sets; and $\mathbf{e_1}$, $\mathbf{e_2}$, and $\mathbf{e_3}$ were vectors for the corresponding residual effects. $\mathbf{X_1}$, $\mathbf{X_2}$, $\mathbf{X_3}$, $\mathbf{Z_1}$, $\mathbf{Z_2}$, $\mathbf{Z_3}$, $\mathbf{W_1}$, $\mathbf{W_2}$, and $\mathbf{W_3}$ were incidence matrices for $\mathbf{b_1}$, $\mathbf{b_2}$, $\mathbf{b_3}$, $\mathbf{u_1}$, $\mathbf{u_2}$, $\mathbf{u_3}$, $\mathbf{p_1}$, $\mathbf{p_2}$, and $\mathbf{p_3}$, respectively. The variance-covariance structure for random effects was as follows:

$$\text{var}\begin{bmatrix}\mathbf{u}_1\\ \mathbf{u}_2\\ \mathbf{u}_3\\ \mathbf{p}_1\\ \mathbf{p}_2\\ \mathbf{p}_3\\ \mathbf{e}_1\\ \mathbf{e}_2\\ \mathbf{e}_3\end{bmatrix} = \begin{bmatrix}\mathbf{g}_{11}\mathbf{A} & \mathbf{g}_{12}\mathbf{A} & \mathbf{g}_{13}\mathbf{A} & 0 & 0 & 0 & 0 & 0 & 0\\ \mathbf{g}_{21}\mathbf{A} & \mathbf{g}_{22}\mathbf{A} & \mathbf{g}_{23}\mathbf{A} & 0 & 0 & 0 & 0 & 0 & 0\\ \mathbf{g}_{31}\mathbf{A} & \mathbf{g}_{32}\mathbf{A} & \mathbf{g}_{33}\mathbf{A} & 0 & 0 & 0 & 0 & 0 & 0\\ 0 & \mathbf{0} & 0 & \boldsymbol{\sigma}^2_{\mathbf{p}_1}\mathbf{I}_{\mathbf{p}_1} & 0 & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & \boldsymbol{\sigma}^2_{\mathbf{p}_2}\mathbf{I}_{\mathbf{p}_2} & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & \boldsymbol{\sigma}^2_{\mathbf{p}_3}\mathbf{I}_{\mathbf{p}_3} & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & 0 & \mathbf{r}_{11} & 0 & 0\\ 0 & 0 & \mathbf{0} & 0 & 0 & 0 & 0 & \mathbf{r}_{22} & 0\\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \mathbf{r}_{33}\end{bmatrix},$$

where $\mathbf{g_{11}}$, $\mathbf{g_{22}}$, and $\mathbf{g_{33}}$ were additive-genetic sire effects for the 3 traits; $\mathbf{g_{12}}$ and $\mathbf{g_{21}}$ were additive genetic covariances for sire effects between traits in small and middle; $\mathbf{g_{13}}$ and $\mathbf{g_{31}}$ were additive-genetic covariances for sire effects between traits in small and high; $\mathbf{g_{23}}$ and $\mathbf{g_{32}}$ were additive-genetic covariances for sire effects between traits in middle and high; $\boldsymbol{\sigma}^2_{\mathbf{p}_1}$, $\boldsymbol{\sigma}^2_{\mathbf{p}_2}$, and $\boldsymbol{\sigma}^2_{\mathbf{p}_3}$ were the variances for permanent environmental cow effects for the 3 traits; $\mathbf{I}_{\mathbf{p}_1}$, $\mathbf{I}_{\mathbf{p}_2}$, and $\mathbf{I}_{\mathbf{p}_3}$ were identity matrices for the cows with records for the 3 traits; and $\mathbf{r_{11}}$, $\mathbf{r_{22}}$, and $\mathbf{r_{33}}$ were residual variances for the 3 traits.

## RESULTS AND DISCUSSION

### *Characteristics of Herd Descriptors*

Averaged $r^2$ for the segment on chromosome 6 was 0.28, and 0.31 within the segment on chromosome 14, both with small SD of 0.01 (Table 3). A small range for intra-herd LD for the 52 herds from the same region indicates a similar breeding history. The slightly higher $r^2$ in the *DGAT1* region compared with the average $r^2$ of pairwise SNP within a 25 kb distance across the whole genome is due to intensive selection on milk production traits in the past decades (Grisart et al., 2002). For the whole genome, Qanbari et al. (2010) reported a slightly larger average $r^2$ value for a German Holstein sub-population, but their analysis based on intensively pre-selected potential bull dams and bull sires. Intra-herd $r_{chr14}{}^2$ within the *DGAT1* segment was moderately correlated with intra-herd $r_{chr6}{}^2$ (0.41; Table 4). Nevertheless, among all herd descriptors, this was one of the strongest associations. The correlation coefficient was slightly larger (0.47) between intra-herd $r_{chr14}{}^2$ and the intra-herd inbreeding coefficient. The correlation of 0.25 between $r_{chr6}{}^2$ and $F$ also reflects the effect of selection on genomic architecture characteristics. In analogy to intra-herd $r^2$-values, intra-herd inbreeding coefficients varied within a small range from 0.04 to 0.05 (Table 3). Hence, inbreeding in the sub-population including only 52 herds from a specific region in the eastern part of Germany does not reflect the broader variation of within-herd inbreeding as identified in the former west Germany (König and Simianer, 2006).

Intra-herd allele frequencies for the SNP located in the *DGAT1* gene were in a broader range from 0.61 to 0.82. Also for this genomic herd descriptor, the coefficient of variation was quite small (0.05). Nevertheless, the allele frequency variations across herds depict the different production trait selection strategies, that is, a fraction of herds with a stronger focus on an allele A (increasing milk yield, but decreasing fat percentage), and other herds favoring the opposite allele B (decreasing milk yield, but increasing fat percentage). The correlation coefficient between $p$ and $r_{chr14}{}^2$ was 0.28. The negative correlation between NRR56 and $F$ (−0.27) was in line with previous findings by Cassell et al. (2003), who argued that increasing inbreeding accumulates harmful recessive alleles in the progenies.

Correlations were weak among the remaining herd descriptors. This is especially true when correlating phenotypic herd descriptors reflecting the herd environment (HS) or the herd management (NRR56) with genomic descriptors. The generally weak to moderate correlations indicate independence among genomic, genetic, and phenotypic characteristics. Low antagonistic correlations were identified between NRR56 with LD descriptors (i.e., −0.14 with $r_{chr6}^2$ and −0.17 with $r_{chr6}^2$). Hence, a better intra-herd nonreturn rate was associated with a lower level of LD. In such a context [i.e., assuming the effect of selection intensity on the LD structure (Pimentel et al., 2013)], antagonistic correlations between production and female fertility traits were especially identified in Holstein herds, which were intensively selected for milk yield over decades (König et al., 2008). In contrast, in the dual-purpose cattle population Deutsches Schwarzbuntes Niederungsrind (the founder population for Holstein, limited selection on milk yield), levels of LD were lower than in the Holstein population, and correlations between MY and fertility were close to zero (Jaeger et al., 2016).

**Table 4**. Correlations among continuous herd descriptors[1]

| | Herd descriptor | | | | | |
|---|---|---|---|---|---|---|
| Item | $r_{chr14}^2$ | *p* | *F* | Sire% | HS | NRR56 |
| $r_{chr6}^2$ | 0.41 | −0.06 | 0.25 | 0.12 | −0.12 | −0.14 |
| $r_{chr14}^2$ | | 0.28 | 0.47 | −0.16 | 0.07 | −0.17 |
| *p* | | | −0.11 | 0.13 | 0.06 | −0.01 |
| *F* | | | | −0.24 | 0.13 | −0.27 |
| Sire% | | | | | 0.18 | −0.05 |
| HS | | | | | | −0.08 |

[1] $r_{chr6}^2 = r^2$ on chromosome 6 between 40 and 60 Mbp; $r_{chr14}^2 = r^2$ on chromosome 14 between 0 and 40 Mbp; *p* = allele frequency of the SNP located in the *DGAT1* gene; *F* = pedigree-based inbreeding coefficient; sire% = percentage of cows with a non-European Union sire; HS = herd size; NRR56 = nonreturn rate after 56 d.

### Genetic Parameters for Test-Day Traits on Continuous Herd Descriptor Scales/Within Herd Descriptor Classes

***Genomic Herd Descriptors.*** With regard to $r_{chr6}^2$, and considering the narrow herd interval from 0.25 to 0.32, heritabilities for test-day traits SCS and Pro% were quite stable (Figure 1A). As expected, among all traits, heritabilities were lowest in the range from 0.10 to 0.12 for the functional trait SCS. Only a minor effect of $r_{chr6}^2$ on heritabilities for SCS was assumed, because identified QTL in this region (Khatkar et al., 2004) indicate intensified selection on milk volume and content traits Pro% and fat%, but not on low heritability SCS. Accordingly, we observed an obvious effect of intra-herd $r_{chr6}^2$ on heritabilities for MY and fat% [i.e., a heritability increase with $r_{chr6}^2 > 0.29$ (Figure 1A)]. Theoretically, larger LD between markers represents that markers are closely linked among each other, and that those markers (same genetic variants) are transmitted from parents to offspring (Falconer and Mackay, 1996). In consequence, the heritability increase for MY and fat% with increasing $r_{chr6}^2$ indicates that markers in herds with higher $r_{chr6}^2$ capture QTL in this region of the genome. This is in line with previous findings (Khatkar et al., 2004), pointing out that this segment on chromosome 6 significantly contributed to milk production traits. Obvious reactions of genetic variances for MY and fat% on LD characteristics might be due to selection. Strong selection intensities on the cow-sire pathway of selection were identified for MY and fat% in worldwide Holstein populations in the past decades (Powell et al., 2003), but cow-sire selection was less intensive for female fertility and SCC (Powell et al., 2003; Miglior et al., 2005).

Heritabilities from the MTRM (Table 5) for test-day traits in the 3 $r_{chr6}^2$ herd classes, small, middle, and high, confirmed the results from the RRM (i.e., larger heritabilities for MY and fat% within high compared with small or middle). For SCS (in analogy to the RRM results), we found lowest heritabilities in the high intra-herd $r_{chr6}^2$ class. However, heritability differences in the different classes were minor, representing the range of standard errors.

Genetic correlations in same test-day traits were close to 1 for neighboring intra-herd $r_{chr6}^2$ values, but decreased with increasing $r_{chr6}^2$ distances (Figure 1B). This was especially the case for MY, but also for SCS. With regard to MY and SCS, genetic correlations between the lowest and the highest $r_{chr6}^2$ herd were 0.35 for both traits. Genetic correlations lower than 0.80 indicate G×E (Robertson, 1959), and a re-ranking of animals when performing in the different genomic herd environments. Also for the MTRM (Table 5), genetic correlations were larger when correlating same traits in small and middle or in middle and high, compared with the more distant herd classes small and high. Because of the relevance of this chromosome 6 segment on milk volume, such effects were assumed for MY, but not for SCS. One argument addresses the general sensitivity of SCS (sensitivity in terms of altering genetic covariances and genetic correlations) in G×E studies. Using classical environmental categories (e.g., geographical regions, sea level, or organic versus conventional), genetic correlations were always close to 1 for test-day production traits, but declined for SCS. This was the case for Holstein (Nauta et al., 2006) as well as for local

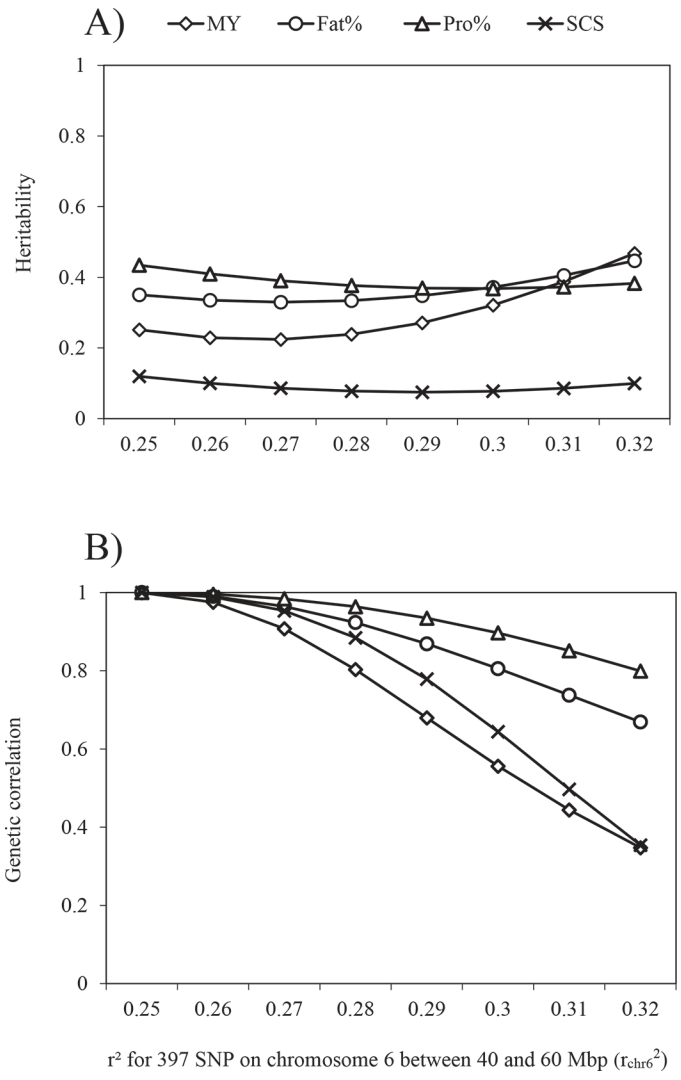


**Figure 1**. Heritabilities (A) and genetic correlations between the minimal level and remaining levels (B) for the genomic herd descriptor "linkage disequilibrium of 397 SNP within 40 to 60 Mbp on chromosome 6 ($r_{chr6}^2$) and the rest of levels" considering the following test-day traits: milk yield (MY), fat percentage (fat%), protein percentage (Pro%), and SCS. Standard errors for heritabilities ranged from 0.053 to 0.112 for MY, from 0.076 to 0.113 for fat%, from 0.085 to 0.101 for Pro%, and from 0.020 to 0.041 for SCS. Standard errors for genetic correlations ranged from 0.001 to 0.062 for MY, from 0.001 to 0.066 for fat%, from 0.001 to 0.073 for Pro%, and from 0.001 to 0.098 for SCS; SE for genetic correlations increased with increasing distance between herd descriptors.

dual-purpose cows (Jaeger et al., 2016). Environmental sensitivity findings for SCS need ongoing investigations.

Considering the extreme 5% tails of herds according to $r_{chr6}^2$, rank correlations between sire EBV were larger for Pro% and fat% compared with MY and SCS (Table 6). Hence, for genetic improvements of Pro% and fat%, same sires are suggested for low and high LD herds. In contrast, for MY and SCS with EBV correlations close

**Table 5**. Heritabilities (diagonal) and genetic correlations (above diagonal) ± SE for same test-day traits in different herd classes defined as small, middle, and high according to genomic herd descriptors[1]

| | Milk yield | | | Fat % | | | Protein % | | | SCS | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | Small | Middle | High | Small | Middle | High | Small | Middle | High | Small | Middle | High |
| $r_{chr6}^2$ | | | | | | | | | | | | |
| Small | 0.24 ± 0.03 | 0.80 ± 0.05 | 0.79 ± 0.06 | 0.35 ± 0.03 | 0.96 ± 0.02 | 0.95 ± 0.02 | 0.40 ± 0.03 | 0.96 ± 0.02 | 0.92 ± 0.03 | 0.09 ± 0.01 | 0.78 ± 0.05 | 0.67 ± 0.07 |
| Middle | | 0.25 ± 0.03 | 0.79 ± 0.06 | | 0.38 ± 0.03 | 0.91 ± 0.03 | | 0.40 ± 0.04 | 0.84 ± 0.04 | | 0.10 ± 0.02 | 0.76 ± 0.09 |
| High | | | 0.27 ± 0.03 | | | 0.44 ± 0.03 | | | 0.38 ± 0.03 | | | 0.07 ± 0.01 |
| $r_{chr14}^2$ | | | | | | | | | | | | |
| Small | 0.27 ± 0.03 | 0.90 ± 0.04 | 0.80 ± 0.04 | 0.32 ± 0.03 | 0.94 ± 0.02 | 0.87 ± 0.02 | 0.39 ± 0.03 | 0.97 ± 0.02 | 0.94 ± 0.02 | 0.08 ± 0.01 | 0.84 ± 0.06 | 0.83 ± 0.07 |
| Middle | | 0.25 ± 0.02 | 0.85 ± 0.05 | | 0.37 ± 0.03 | 0.93 ± 0.03 | | 0.36 ± 0.04 | 0.92 ± 0.03 | | 0.10 ± 0.01 | 0.78 ± 0.07 |
| High | | | 0.36 ± 0.03 | | | 0.44 ± 0.03 | | | 0.33 ± 0.03 | | | 0.07 ± 0.01 |
| *p* | | | | | | | | | | | | |
| Small | 0.21 ± 0.03 | 0.94 ± 0.04 | 0.89 ± 0.04 | 0.33 ± 0.03 | 0.98 ± 0.02 | 0.93 ± 0.03 | 0.44 ± 0.04 | 0.95 ± 0.02 | 0.93 ± 0.03 | 0.09 ± 0.01 | 0.90 ± 0.07 | 0.80 ± 0.07 |
| Middle | | 0.23 ± 0.03 | 0.76 ± 0.05 | | 0.32 ± 0.03 | 0.94 ± 0.02 | | 0.36 ± 0.03 | 0.91 ± 0.03 | | 0.09 ± 0.01 | 0.84 ± 0.05 |
| High | | | 0.32 ± 0.03 | | | 0.37 ± 0.03 | | | 0.40 ± 0.03 | | | 0.07 ± 0.01 |

[1] $r_{chr6}^2 = r^2$ on chromosome 6 between 40 and 60 Mbp; $r_{chr14}^2 = r^2$ on chromosome 14 between 0 and 40 Mbp; $p$ = allele frequency of the SNP located in the *DGAT1* gene.

**Table 6**. Rank correlations for estimated sire test-day trait breeding values (the 681 sires with daughters) between the lower 5th and upper 95th percentiles for herd descriptors

| Herd descriptor | Test-day trait | | | |
|---|---|---|---|---|
| | Milk yield | Fat % | Protein % | SCS |
| $r^2$ on chromosome 6 between 40 and 60 Mbp ($r_{chr6}^2$) | 0.80 | 0.96 | 0.99 | 0.84 |
| $r^2$ on chromosome 14 between 0 and 40 Mbp ($r_{chr14}^2$) | 0.78 | 0.94 | 1.00 | 0.88 |
| Allele frequency of the SNP located in the *DGAT1* gene ($p$) | 0.83 | 0.92 | 0.95 | 0.87 |
| Pedigree based inbreeding coefficient ($F$) | 0.73 | 0.92 | 0.97 | 0.92 |
| Percentage of cows with a non-European Union sire (sire%) | 0.58 | 0.99 | 0.95 | 0.80 |
| Herd size (HS) | 0.80 | 0.95 | 0.97 | 0.61 |
| Nonreturn rate after 56 d (NRR56) | 0.82 | 0.93 | 0.93 | 0.69 |

to 0.80, specific sires should be selected for specific genomic herd architectures.

With regard to heritabilities for test-day traits on the continuous to $r_{chr14}^2$ scale (Figure 2A), again we identified lowest values in a narrow range (0.11–0.13) for SCS. In analogy with the $r_{chr6}^2$ descriptor, heritabilities for MY and fat% gradually increased with increasing levels for $r_{chr14}^2$. Among all test-day traits, the highest heritability of 0.53 for fat% was observed at the highest $r_{chr14}^2$ level. The increase of heritabilities for MY and fat% with increasing intra-herd $r_{chr14}^2$, and also the larger extent of LD for the chromosome 14 segment compared with the segment on chromosome 6, might be due to the pronounced effect of the *DGAT1* gene. The segment from 1,463,676 to 2,117,455 bp on chromosome 14 explained 41.4% of the total SNP variance for fat% (van Binsbergen et al., 2012), reflecting the variance proportion (31%) directly explained by the *DGAT1 K232A* polymorphism (Grisart et al., 2002). Heritabilities for Pro% slightly decreased with increasing intra-herd $r_{chr14}^2$. In Holstein populations, allele substitution effects for the *DGAT1 K232A* polymorphism on Pro% were quite small, in the range from 0.05% (Kühn et al., 2004) to 0.08% (Thaller et al., 2003). With regard to the MTRM, heritabilities for MY and fat% were also largest in the herd class high (Table 5). Also the slight heritability decrease for Pro% from small to high, and the quite constant SCS heritabilities for all 3 herd classes, confirmed RRM estimates.

For the RRM applications (Figure 2B), genetic correlations of 0.63 for fat%, of 0.67 for SCS, and of 0.37 for MY, were quite small between the minimum and maximum $r_{chr14}^2$ levels, suggesting a gradually changing genetic background of the 3 test-day traits in dependency of $r_{chr14}^2$. In general, with RRM and time dependent covariates, estimates at the "extreme ends of the time scale" differed from the remaining scale levels and were associated with large standard errors (Gernand and König, 2014). However, in the present study for the $r_{chr14}^2$ descriptor, standard errors of genetic correlations for fat%, Pro%, and MY were generally smaller than 0.07. For Pro%, not only did the heritability curve differ from the corresponding estimates for fat% and MY (Figure 2A), but also the genetic correlations between different $r_{chr14}^2$ levels were substantially larger (Figure 2B). With regard to genetic correlations from the MTRM (Table 5), and with regard to correlations between sire EBV (Table 6), estimates confirmed the general trends from the RRM, but were substantially larger for all traits (closer to 1) and exactly 1.00 for Pro% (EBV correlations).

Heritability pattern for test-day traits on the continuous allele frequency ($p$) scale (Figure 3a) are in close agreement with the corresponding trait curves on the $r_{chr14}^2$ scale. This is the logical consequence, because we assume the effect of *DGAT1* selection strategies on $r_{chr14}^2$, as indicated via the moderate correlation of 0.28 between both herd descriptors. Again, heritabilities for SCS were quite constant in the range from 0.09 to 0.11 for all intra-herd allele frequencies, and heritabilities for MY and fat% increased with increasing $p$. In the present study, the minor allele frequency for the SNP marker *ARS-BFGL-NGS-4939* averaged across all herds was 0.31, and therefore larger than in Italian Holsteins (Minozzi et al., 2013). The larger minor allele frequency indicate more diverse selection strategies in German herds: some farms focus on genetic improvements of MY, whereas other farm have a stronger focus on the content trait fat%. Hence, genetic parameters of both traits MY and fat% altered on the continuous $p$ scale. Significant effects of minor allele frequencies for the marker *ARS-BFGL-NGS-4939* on allele substitution effects for production traits were identified in Italian (Minozzi et al., 2013) and in Chinese Holstein cattle populations (Jiang et al., 2010). In consequence, allele frequencies affect genetic variances. Heritabilities from the MTRM for fat% and MY were larger in the high classes compared with the small classes, but we detected only minor differences between small and middle (Table 5). Again, and in agreement with the

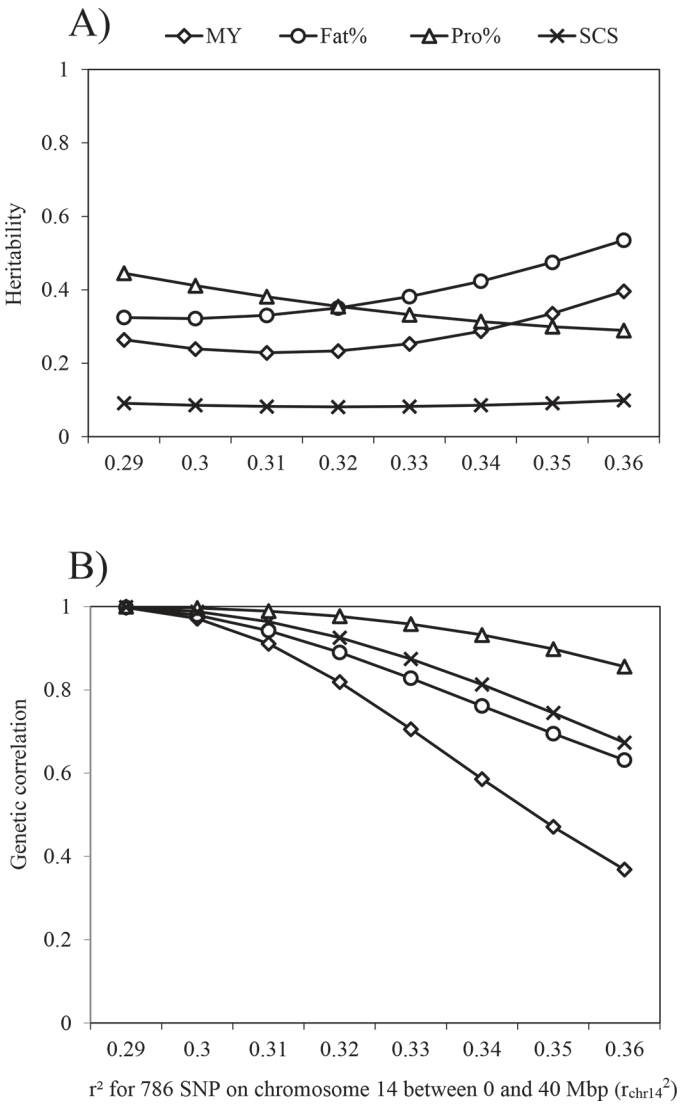


**Figure 2**. Heritabilities (A) and genetic correlations between the minimal level and remaining levels (B) for the genomic herd descriptor "linkage disequilibrium of 786 SNP between 0 and 40 Mbp on chromosome 14 ($r_{chr14}^2$)" considering the following test-day traits: milk yield (MY), fat percentage (fat%), protein percentage (Pro%), and SCS. Standard errors for heritabilities ranged from 0.054 to 0.110 for MY, from 0.074 to 0.113 for fat%, from 0.078 to 0.100 for Pro%, and from 0.021 to 0.041 for SCS. Standard errors for genetic correlations ranged from 0.001 to 0.059 for MY, from 0.001 to 0.063 for fat%, from 0.001 to 0.079 for Pro%, and from 0.001 to 0.065 for SCS; SE for genetic correlations increased with increasing distance between herd descriptors.

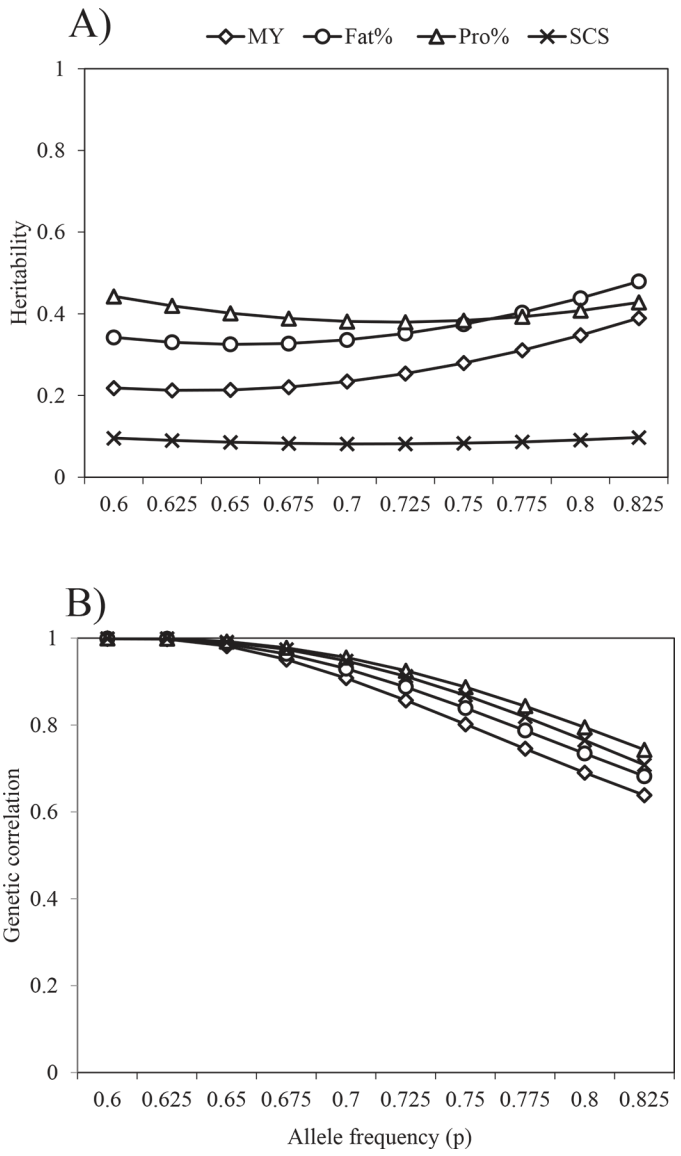


**Figure 3**. Heritabilities (A) and genetic correlations between the minimal level and remaining levels (B) for the genomic herd descriptor "allele frequency of the marker *ARS-BFGL-NGS-4939* (p)" considering the following test-day traits: milk yield (MY), fat percentage (fat%), protein percentage (Pro%), and SCS. Standard errors for heritabilities ranged from 0.047 to 0.090 for MY, from 0.070 to 0.105 for fat%, from 0.080 to 0.097 for Pro%, and from 0.020 to 0.031 for SCS. Standard errors for genetic correlations ranged from 0 to 0.063 for MY, from 0 to 0.056 for fat%, from 0.001 to 0.058 for Pro%, and from 0.001 to 0.116 for SCS; SE for genetic correlations increased with increasing distance between herd descriptors.

RRM estimates, heritabilities for Pro% and SCS were quite constant for the 3 herd classes (Table 5).

The shape of genetic correlation curves (Figure 3B) was identical for all test-day traits. Genetic correlations were minimal when correlating the respective traits from intra-herd allele frequencies in greatest distance (i.e., traits recorded at $P = 0.6$ and at $P = 0.825$). However, genetic correlations for the $p$ descriptor were generally larger compared with the LD descriptors. In agreement with the $r_{chr14}^2$ descriptor, the lowest genetic correlations were estimated for MY and the largest for Pro%. Genetic correlations from the MTRM (Table 5) and the correlations between sire EBV (Table 6) were larger than 0.80 for all test-day traits.

***Genetic Herd Descriptors.*** Among all herd descriptors, we identified the largest association between $r_{chr14}^2$ and $F$ (correlation coefficient of 0.47). In consequence, similarities of heritability curves were identified for both continuous herd descriptors $r_{chr14}^2$ and $F$. Heritabilities were quite constant (0.06 to 0.10) for SCS on a low $F$ level, heritabilities decreased with increasing $F$ for Pro%, and heritabilities for MY and fat% increased within the range from moderate to highest intra-herd inbreeding coefficients (Figure 4a). However, also for MY and fat%, the heritability was largest for the lowest intra-herd inbreeding coefficient. Theoretically, in a long-term perspective, intensive selection accumulates inbreeding, and reduces genetic variation within populations (Falconer and Mackay, 1996). In the present study, largest heritabilities for MY, fat%, and Pro% for herds with lowest inbreeding are in line with those theoretical expectations. Regarding intra-herd inbreeding coefficients in the present study, only minor herd differences existed, in a narrow range from $F = 0.034$ to $F = 0.052$. Increase of $F$ did not influence heritabilities for SCS, reflecting the nonsignificant inbreeding depressions for SCS on phenotypic scales in older (Miglior et al., 1992) as well as in more recent studies (Thompson et al., 2000). As found for genomic descriptors, also for pedigree-based $F$, heritability estimates from the MTRM confirmed the RRM trends, but (apart from MY) estimates in the 3 different $F$ classes only differed marginally (Table 7).

Genetic correlations in MY decreased when considering herds with large intra-herd $F$ differences (Figure 4B), supporting genetic correlation estimates from the MTRM (Table 7), and also reflecting the sire EBV correlation for MY (Table 6). Inbreeding within herds accumulated due to the strong selection focus on MY in past decades (König and Simianer, 2006), which might explain the pronounced effects of $F$ on genetic covariance and genetic correlation estimates for MY. Among all herd descriptors, only for the intra-herd inbreeding scale, genetic correlation estimates were largest for SCS, but associated with largest SE (Figure 4B). Nevertheless, genetic correlations for SCS across different herd classes were also quite large with $r_g > 0.80$ (Table 7). Quantitative genetic parameters for SCS from the present analysis might be surprising because, in general, low heritability functional traits react more sensitively to environmental or herd descriptor alterations compared with production traits. Nevertheless, SCS seems to be a specific functional trait. Generally, in genomic predictions, and also when following deterministic equations (Goddard, 2009), reliabilities of genomic breeding values increased with increasing trait heritability. However, in German national genomic evaluations for Holstein sires, among all traits, prediction accuracies are largest for low heritability SCS.

In addition to intra-herd inbreeding coefficients, we estimated genetic parameters in dependency of intra-herd pedigree based relationships. As expected, results were similar for both descriptors, because relationships among parents directly reflect inbreeding in offspring. Also for the intra-herd genetic relationships, genetic

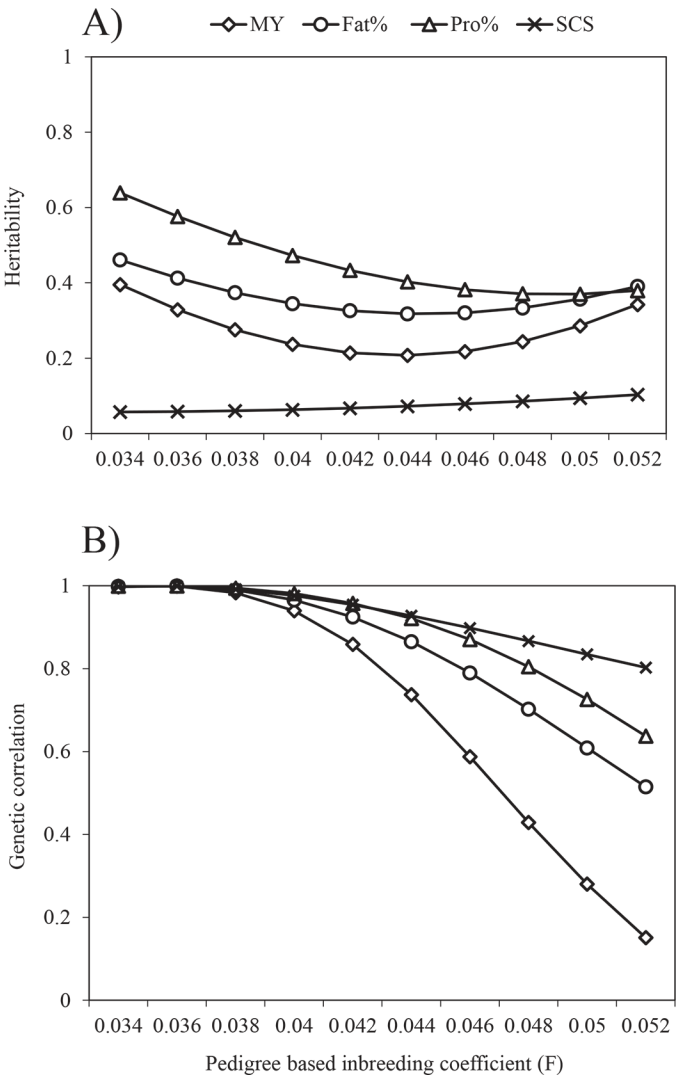


**Figure 4**. Heritabilities (A) and genetic correlations between the minimal level and the remaining levels (B) for the genetic herd descriptor "inbreeding coefficient (F)" considering the following test-day traits: milk yield (MY), fat percentage (fat%), protein percentage (Pro%), and SCS. Standard errors for heritabilities ranged from 0.048 to 0.115 for MY, from 0.069 to 0.117 for fat%, from 0.078 to 0.147 for Pro%, and from 0.019 to 0.037 for SCS. Standard errors for genetic correlations ranged from 0.001 to 0.050 for MY, from 0.01 to 0.062 for fat%, from 0.001 to 0.069 for Pro%, and from 0.001 to 0.306 for SCS; SE for genetic correlations increased with increasing distance between herd descriptors.

**Table 7**. Heritabilities (diagonal) and genetic correlations (above diagonal) for same test-day traits in different herd classes defined as small, middle, and high according to genetic and phenotypic herd descriptors[1]

| | Milk yield | | | Fat % | | | Protein % | | | SCS | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | Small | Middle | High | Small | Middle | High | Small | Middle | High | Small | Middle | High |
| *F* | | | | | | | | | | | | |
| Small | 0.31 ± 0.03 | 0.92 ± 0.04 | 0.74 ± 0.06 | 0.41 ± 0.03 | 0.94 ± 0.02 | 0.88 ± 0.02 | 0.41 ± 0.03 | 0.96 ± 0.02 | 0.96 ± 0.02 | 0.08 ± 0.01 | 0.84 ± 0.05 | 0.83 ± 0.11 |
| Middle | | 0.22 ± 0.03 | 0.78 ± 0.04 | | 0.35 ± 0.03 | 0.94 ± 0.02 | | 0.39 ± 0.03 | 0.99 ± 0.02 | | 0.10 ± 0.01 | 0.85 ± 0.07 |
| High | | | 0.27 ± 0.03 | | | 0.36 ± 0.03 | | | 0.36 ± 0.03 | | | 0.07 ± 0.01 |
| Sire% | | | | | | | | | | | | |
| Small | 0.27 ± 0.03 | 0.85 ± 0.05 | 0.82 ± 0.04 | 0.35 ± 0.03 | 0.97 ± 0.02 | 0.96 ± 0.02 | 0.39 ± 0.03 | 0.93 ± 0.02 | 0.94 ± 0.02 | 0.10 ± 0.02 | 0.87 ± 0.07 | 0.84 ± 0.08 |
| Middle | | 0.24 ± 0.03 | 0.89 ± 0.04 | | 0.35 ± 0.03 | 0.98 ± 0.01 | | 0.39 ± 0.03 | 0.95 ± 0.02 | | 0.07 ± 0.01 | 0.82 ± 0.06 |
| High | | | 0.27 ± 0.03 | | | 0.34 ± 0.03 | | | 0.42 ± 0.03 | | | 0.10 ± 0.01 |
| HS | | | | | | | | | | | | |
| Small | 0.27 ± 0.03 | 0.85 ± 0.04 | 0.82 ± 0.05 | 0.35 ± 0.04 | 0.88 ± 0.04 | 0.85 ± 0.04 | 0.39 ± 0.04 | 0.95 ± 0.03 | 0.91 ± 0.03 | 0.08 ± 0.02 | 0.88 ± 0.09 | 0.75 ± 0.08 |
| Middle | | 0.21 ± 0.03 | 0.83 ± 0.04 | | 0.32 ± 0.03 | 0.93 ± 0.02 | | 0.40 ± 0.04 | 0.93 ± 0.02 | | 0.09 ± 0.02 | 0.88 ± 0.06 |
| High | | | 0.35 ± 0.02 | | | 0.35 ± 0.03 | | | 0.42 ± 0.03 | | | 0.12 ± 0.01 |
| NRR56 | | | | | | | | | | | | |
| Small | 0.32 ± 0.03 | 0.80 ± 0.06 | 0.76 ± 0.04 | 0.35 ± 0.03 | 0.95 ± 0.02 | 0.94 ± 0.02 | 0.38 ± 0.03 | 0.91 ± 0.03 | 0.91 ± 0.03 | 0.07 ± 0.01 | 0.82 ± 0.05 | 0.72 ± 0.08 |
| Middle | | 0.27 ± 0.03 | 0.81 ± 0.05 | | 0.32 ± 0.03 | 0.94 ± 0.03 | | 0.42 ± 0.04 | 0.90 ± 0.03 | | 0.09 ± 0.01 | 0.80 ± 0.06 |
| High | | | 0.31 ± 0.02 | | | 0.33 ± 0.03 | | | 0.45 ± 0.03 | | | 0.11 ± 0.01 |
| Random[2] | 0.26 ± 0.03 | 0.94 ± 0.06 | 0.94 ± 0.06 | 0.34 ± 0.02 | 0.95 ± 0.02 | 0.94 ± 0.02 | 0.40 ± 0.03 | 0.92 ± 0.04 | 0.93 ± 0.04 | 0.09 ± 0.01 | 0.87 ± 0.08 | 0.89 ± 0.07 |
| | | 0.25 ± 0.03 | 0.93 ± 0.08 | | 0.33 ± 0.03 | 0.95 ± 0.03 | | 0.39 ± 0.03 | 0.92 ± 0.03 | | 0.09 ± 0.01 | 0.86 ± 0.08 |
| | | | 0.25 ± 0.03 | | | 0.33 ± 0.02 | | | 0.40 ± 0.03 | | | 0.09 ± 0.01 |

[1]*F* = pedigree-based inbreeding coefficient; sire% = percentage of cows with a non-European Union sire; HS = herd size; NRR56 = nonreturn rate after 56 d.
[2]Random herd number allocation: estimates are means from 50 replicates, along with corresponding SD.

correlation were largest for SCS, with $r_g > 0.92$ for all pairwise combinations from the RRM application. In agreement with the intra-herd inbreeding coefficients, genetic correlations for MY substantially decreased with increasing intra-herd relationship differences. For example, the genetic correlation was only 0.10 when correlating MY between herds with the lowest intra-herd relationship coefficient (0.09) and herds with the largest intra-herd relationship coefficient (0.14).

For the sire% herd descriptor, heritability curves were different for fat% and Pro% (Figure 5A). Heritabilities for fat% slightly decreased from 0.38 to 0.25 with the increase of intra-herd sire%, but heritabilities for Pro% increased from 0.39 to 0.52. Only by trend, the MTRM heritabilities for distinct classes (Table 7) reflect the RRM heritability curve pattern for fat% and Pro%. Also for this herd descriptor, heritabilities for SCS were in a low and constant range over all sire% levels.

Especially genetic correlations for MY were extremely low for substantial differences in sire herd structures (Figure 5B). The genetic correlation in MY between the 0% of non-EU sires herds with the 39% of non-EU sires was only 0.26. Such an effect of the genetic structure of herds on genetic correlations reflects the findings from our previous studies as explained via Table 1. The genetic composition of herds influenced genetic correlations in yield traits, and it was not only the herd location (east versus west), which contributed to G×E. Sires from the United States used in German AI programs were intensively selected elite sires with mostly outstanding genetic values for MY and protein yield. In contrast, sires from German origin also represented young bulls from progeny testing programs with partly extremely low EBV, or proven cow sires with only average genetic values. In the era of genomic selection allowing intensive pre-selection among young sires, genetic levels of all sire origins substantially improved. Hence, in future analyses in this regard, we hypothesize only minor effects of sire herd structures on genetic covariance component estimates.

Obvious decreases in genetic correlation estimates with increasing herd distances for sire% were identified for MY and for SCS (Figure 5B). The MY and SCS genetic correlation decrease is in line with results for sire breeding value correlations (Table 6), and with estimates from the MTRM (Table 7). As outlined above, G×E for SCS were always obvious in previous studies when grouping herds according to classical environmental or farm management descriptors (Hayes et al., 2003; Mulder et al., 2004; van Binsbergen et al., 2012). Generally, such dairy cattle farms applying an improved herd management have a stronger focus on proven sires from foreign countries with outstanding genetic values (König and Simianer, 2006).

***Phenotypic Herd Descriptors.*** Increasing herd size was associated with a SCS heritability increase (Figure 6A). Hammond (1947) suggested superior environments for animal selection, because in superior environments, animals can fully express their true genetic potential. Henderson (1964) recommended optimal environments in terms of herd husbandry and management conditions for progeny testing in AI programs. König et al. (2005) identified improved intra-herd feeding, husbandry, and management conditions in large-scale farms. Hence, a large German dairy cattle farm might represent such a superior environment, allowing clear genetic differentiation. However, regarding genetic differentiation in functional health or health indicator traits (e.g., SCS), Schierenbeck et al. (2011) found extreme daughter yield deviations, larger heritabilities, and larger additive-genetic variances in herds where the cows' immune system is under challenge. Larger heritabilities for MY in large-scale herds (Figure 6A, Table 7) correspond with previous findings in other German federal states for protein yield (König et al., 2005).

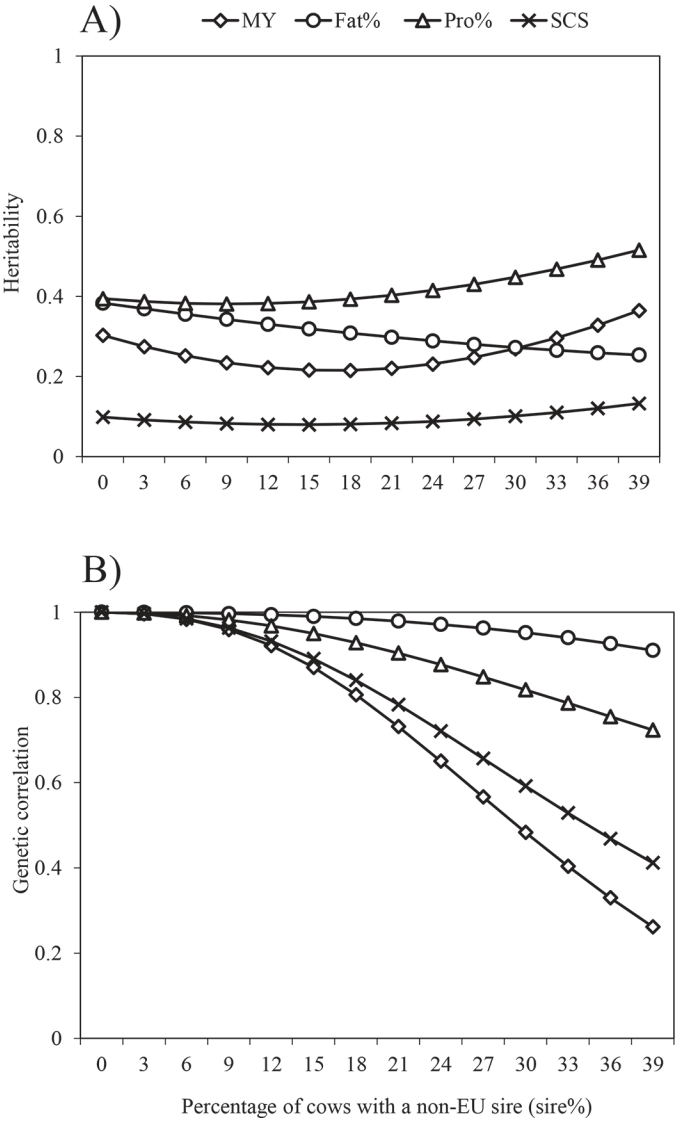


**Figure 5**. Heritabilities (A) and genetic correlations between the minimal level and remaining levels for the genetic herd descriptor "percentage of cows with a non-EU sire (sire%)" (B) considering the following test-day traits: milk yield (MY), fat percentage (fat%), protein percentage (Pro%), and SCS. Standard errors for heritabilities ranged from 0.042 to 0.083 for MY, from 0.055 to 0.070 for fat%, from 0.070 to 0.102 for Pro%, and from 0.017 to 0.040 for SCS. Standard errors for genetic correlations ranged from 0.001 to 0.048 for MY, from 0.001 to 0.054 for fat%, from 0.001 to 0.051 for Pro%, and from 0.001 to 0.075 for SCS; SE for genetic correlations increased with increasing distance between herd descriptors.

Genetic correlations from the RRM between the smallest and the largest herd were quite low for MY (0.27), and even lower for SCS (0.16) (Figure 6B). Accordingly, genetic correlations in both traits between the distant herd classes small and high were larger compared with the more similar herd classes small and middle, or middle and high (Table 7). However, substantially lower genetic correlations for MY and SCS from the RRM compared with the MTRM needs further clarification. Partly surprising or overestimated genetic (co)variance components were identified in previous RRM studies when using continuous time scales (Gernand and König, 2014). Explanations addressed the limited number of observations at the extreme ends of the time scale, or the covariance function used for longitudinal data analyses. Due to the substantial differences in German farm types (i.e., on one hand the small family farm and on the other hand large-scale industrial types), data stratification according to herd sized contributed to G×E. In contrast, in other countries, such as Australia (Hayes et al., 2003), genetic correlations were close to 1 when grouping herds according to herd size.

Remaining test-day production trait heritabilities continuously increased from the middle of the NRR56 scale (NRR56 = 0.50) toward best fertility herds (NRR56 = 0.69). A high fertility status of herds reflects improved farm management, contributing to optimized genetic differentiation (Schierenbeck et al., 2011). For MY and fat%, heritabilities were quite large in herds with extremely poor nonreturn rates (Figure 7A). This was also the case for the MTRM applications (Table 7). Some authors (e.g., König et al., 2008) were very critical of the trait nonreturn rate, because biased genetic (co)variance components might be due to poor data quality (e.g., the utilization of natural service bulls or insufficient trait recording).

The shape of genetic correlation curves, and genetic correlations between the worst NRR56 herd with re-

maining herds, were quite similar for all test-day traits (Figure 7B). Lowest genetic correlations were found for SCS, and were largest for fat% and Pro%. In studies aiming on phenotypic herd descriptors (Jaeger et al., 2016), low heritability functional traits were more susceptible to G×E compared with high heritability production traits. Accordingly, correlations among sire EBV reflect obvious environmental sensitivity for SCS and MY (Table 7).

For the random herd descriptor and the MTRM application, genetic correlations in same traits from different random herd classes were larger than 0.90 for

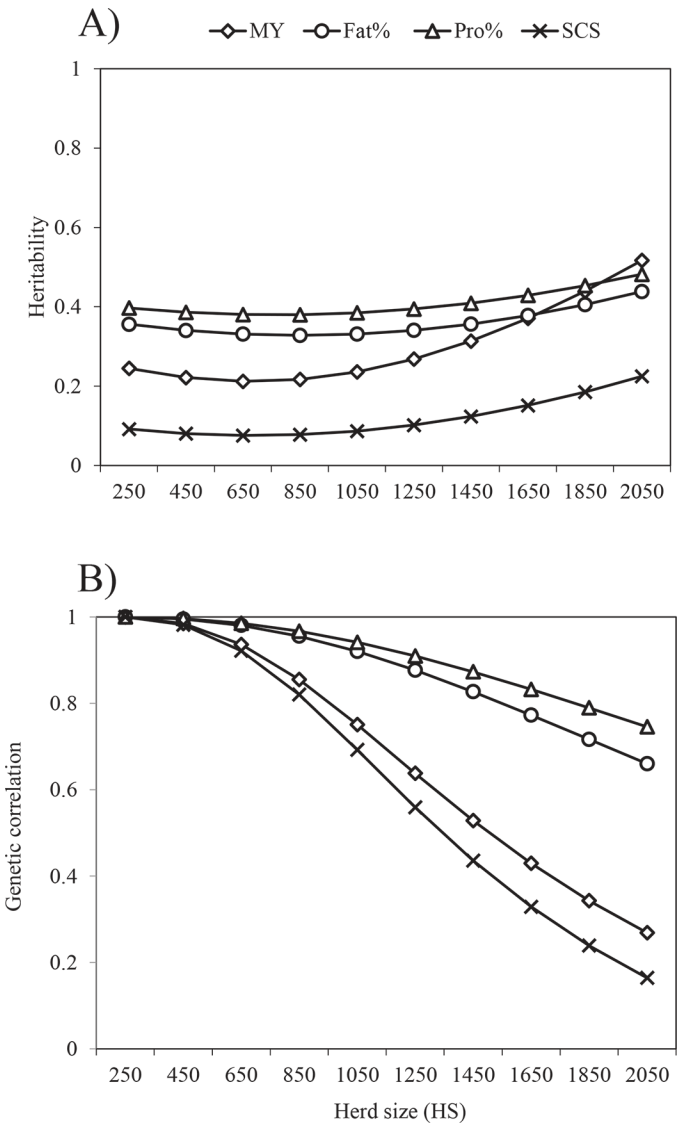


**Figure 6**. Heritabilities (A) and genetic correlations between the minimal level and remaining levels for the phenotypic herd descriptor "herd size (HS)" (B) considering the following test-day traits: milk yield (MY), fat percentage (fat%), protein percentage (Pro%), and SCS. Standard errors for heritabilities ranged from 0.047 to 0.111 for MY, from 0.069 to 0.097 for fat%, from 0.079 to 0.105 for Pro%, and from 0.019 to 0.057 for SCS. Standard errors for genetic correlations ranged from 0.001 to 0.039 for MY, from 0.001 to 0.048 for fat%, from 0.001 to 0.046 for Pro%, and from 0.001 to 0.048 for SCS; SE for genetic correlations increased with increasing distance between herd descriptors.

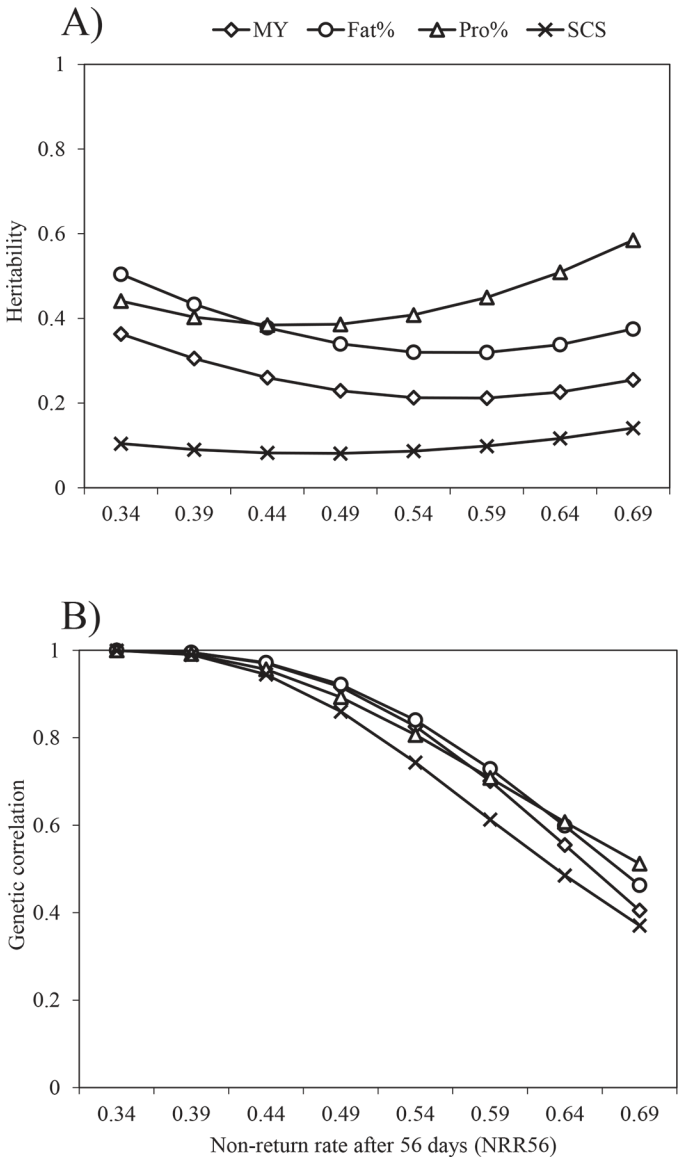


**Figure 7**. Heritabilities (A) and genetic correlations between the minimal level and remaining levels for the phenotypic herd descriptor "nonreturn rate after 56 days (NRR56)" (B) considering the following test-day traits: milk yield (MY), fat percentage (fat%), protein percentage (Pro%), and SCS. Standard errors for heritabilities ranged from 0.051 to 0.088 for MY, from 0.075 to 0.117 for fat%, from 0.089 to 0.137 for Pro%, and from 0.021 to 0.045 for SCS. Standard errors for genetic correlations ranged from 0.001 to 0.048 for MY, from 0.001 to 0.046 for fat%, and from 0.001 to 0.083 for SCS; SE for genetic correlations increased with increasing distance between herd descriptors.

**Table 8**. Akaike information criteria (AIC) and Schwarz Bayesian information criteria (BIC) for random regression models with different continuous herd descriptors[1]

| | Milk yield | | Fat % | | Protein % | | SCS | |
|---|---|---|---|---|---|---|---|---|
| Descriptor | AIC | BIC | AIC | BIC | AIC | BIC | AIC | BIC |
| $r_{chr6}^2$ | 986,388 | 986,440 | −102,382 | −102,329 | −582,192 | −582,139 | 395,158 | 395,211 |
| $r_{chr14}^2$ | 986,371 | 986,423 | −102,382 | −102,330 | −582,198 | −582,146 | 395,163 | 395,216 |
| *p* | 986,377 | 986,423 | −102,381 | −102,328 | −582,204 | −582,152 | 395,163 | 395,215 |
| *F* | 986,361 | 986,413 | −102,385 | −102,332 | −582,214 | −582,152 | 395,166 | 395,218 |
| Sire% | 986,336 | 986,389 | −102,376 | −102,333 | −582,211 | −582,159 | 395,150 | 395,202 |
| HS | 986,295 | 986,348 | −102,393 | −102,340 | −582,212 | −582,159 | 395,087 | 395,140 |
| NRR56 | 986,365 | 986,417 | −102,410 | −102,357 | −582,242 | −582,189 | 395,150 | 395,210 |

[1]$r_{chr6}^2 = r^2$ on chromosome 6 between 40 and 60 Mbp; $r_{chr14}^2 = r^2$ on chromosome 14 between 0 and 40 Mbp; *p* = allele frequency of the SNP located in the *DGAT1* gene; *F* = pedigree-based inbreeding coefficient; sire% = percentage of cows with a non-European Union sire; HS = herd size; NRR56 = nonreturn rate after 56 d.

MY, fat%, and Pro% (Table 7). Interestingly, also for a random herd allocation, lowest correlations (but also close to 0.90) were estimated for SCS. Also from the RRM, large genetic correlations were identified. Only by trend, genetic correlations were larger for neighboring random herd numbers compared with herd numbers in greater distance. This might be an artifact of random regression modeling with the chosen polynomial structure.

Interestingly, RRM performed better when using continuous genetic or phenotypic herd descriptors compared with genomic herd descriptors. In this regard, and for all test-day traits, Akaike information criteria (**AIC**) and Schwarz Bayesian information criteria (**BIC**) were smaller for *F*, sire%, HS, and NRR56 compared with $r_{chr6}^2$, $r_{chr14}^2$, or *p* (Table 8). The AIC and BIC calculations take the number of model parameters into account. Hence, such evaluation criteria also allow RRM–MTRM model comparisons for same traits. Both AIC and BIC were always smaller for the MTRM, indicating superiority of simpler and more robust genetic-statistical modeling via MTRM for such type of data combined with the herd descriptors used.

## CONCLUSIONS

Although linkage disequilibrium and allele frequencies are characteristics for different populations, breeds, and species, intra-herd means from Holstein cows varied on the genomic scale ($r_{chr6}^2$, $r_{chr14}^2$, *p*). Genomic herd differences might be due to different selection strategies after reunification in 1990. For MY, being the trait under intensive selection for decades, heritabilities increased with increasing $r_{chr6}^2$, $r_{chr14}^2$, and *p*. Furthermore, an extremely small and a high intra-herd inbreeding coefficient, a high percentage of sires from non-EU countries, a large herd size, and a high intra-herd NRR56 contributed to MY heritability increases. Heritabilities were always lowest and quite constant for SCS in dependency of intra-herd variations. Genetic correlations were lower than 0.80 when considering herds "in great descriptor distance," especially for MY and SCS. Generally, results from the MTRM were in agreement with those from the RRM, but less extreme (closer to 1). Correlations among sire EBV reflected the genetic correlation estimates, suggesting specific sires for specific herd structures. In the present study, we identified the effect of phenotypic, genetic, and genomic herd compositions on genetic correlations. From a practical perspective, we suggest utilization of a broad herd pattern when designing cow calibration groups to avoid possible G×E due to specific herd architectures.

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## 2.9. Original research paper 9

**Yin**, **T.**, and S. König:

Genomics for phenotype prediction and management purposes.

Animal Frontiers 2016; 6:65–72

# Genomics for phenotype prediction and management purposes

**T. Yin and S. König**

Department of Animal Breeding, University of Kassel, 37213 Witzenhausen, Germany

## Implications

- Pre-assuming accuracies of genomic breeding values larger than 0.7 for a moderate heritability production trait, and larger than 0.5 for a low heritability functional trait, additional profit from genotyping female calves or heifers compensates costs for genotyping in commercial herds.
- Herd management will be improved by including SNP information into electronically mating software, e.g., through the exploitation of non-additive genetic effects and via controlling of inbreeding and genetic relationships.
- Random forest methodology can infer binary disease phenotypes in validation sets with moderate accuracy, also for a small number of diseased genotyped animals in training sets.
- Genomic random regression models can be used to predict genomic breeding values for animals without phenotypes in, e.g., harsh environments.

**Key words:** cow calibration groups, prediction of phenotypes, within-herd selection strategies

## Introduction

### *General genomic selection applications in breeding programs*

Genomic selection (**GS**) as theoretically introduced by Meuwissen et al. (2001) has been successfully implemented worldwide in dairy cattle breeding programs reflecting large populations, i.e., Holsteins, Fleckvieh, Jersey, and Brown Swiss, and has revolutionized conventional breeding schemes. Revolution of dairy cattle breeding programs via GS was the logical consequence due to the obvious possibilities in shortening generation intervals combined with highly accurate genomic breeding values, increased selection intensities, and prevention of preferential treatment of bull dams. Improvement of those breeding program characteristics simultaneously contributed to increased economic gain (König et al., 2009; König and Swalve, 2009). Prerequisite for highly accurate genomic breeding values is the implementation of large sire calibration groups including bulls with highly reliable conventional breeding values, as established within the EuroGenomics consortium (Lund et al., 2010). Typically, calibration group sires are genotyped with high-density SNP-chip panels, and estimated additive-genetic values for SNP or haplotype effects are transmitted to male selection candidates (young bulls) from the broad population.

Further GS applications focus on genotyping females with the objectives i) to improve within-herd selection strategies using low-density 10K SNP-chip panels and ii) to predict phenotypes and to explain causal mutations using high-density or even whole-genome sequence data. Objective i addresses the evaluation of on-farm selection strategies combined with the utilization of reproduction technologies while still using "SNP-equations" from a bull calibration group. Objective ii implies relating genotypes directly to phenotypes, along with an evaluation of statistical methodology for phenotype prediction, with genome-wide association studies and with studies on genotype by environment (farm) interactions. As outlined, a major focus is placed on dairy cattle, but at specific points, applications to other species will be discussed as well.

## Improving the Herd Management Using Genomic Information

### *Improving within-herd selection*

Genotyping of female animals has rarely been considered in genomic breeding program designs (e.g., Schaeffer, 2006), mainly due to low selection pressure, high replacement rates, and minor impact of the cow dam pathway on genetic gain (Van Tassel and Van Vleck, 1991). However, with the availability of inexpensive low density (**LD**) SNP chips, interest in genotyping of females has strongly increased, especially for improvements of intra-herd selections on commercial dairy cattle farms (Wiggans et al., 2012). Genotyping of female calves and heifers in combination with the use of reproductive technologies like embryo transfer (**ET**) or sexed semen promises substantial decreases in generation intervals while simultaneously increasing selection intensities (Chesnai, 2012; Schefers and Weigel, 2012). Moreover, a genomic breeding value is an unbiased selection instrument and free from preferential treatment (Pryce and Hayes, 2011).

The computer package *SIG-R* (Pimentel and König, 2012) was used to assess the impact of genotyped females on selection response for single traits and on monetary genetic gain for different within-herd breeding strategies. The computer package *SIG-R* was developed to combine genomic and phenotypic information sources via selection index methodology based on the theoretical approach by Dekkers (2007). For this purpose, a dairy cattle herd with 200 milking cows was assumed. Ninety heifer calves (under 12 mo) and 80 heifers (12 to 24 mo) represented potential replacement candidates of the herd. The replacement rate was fixed to 25% in the basic runs for each scenario, but further on, varied within the range from 25 to 40% in increments of 5%. Generation intervals were defined according to the selection structure of the different scenarios, by


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**Table 1. Phenotypic information sources with respect to the production (MY) and functional trait (FL) as used (indicated with a "+") for the different within-herd breeding scenarios. A "g" indicates a genomic breeding value for the given trait.**

| | Scenario | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | | II | | III | | IV | | V | | VI | |
| | MY | FL | MY | FL | MY | FL | MY | FL | MY | FL | MY | FL |
| Selection candidate | + | | g | g | | | g | g | | | g | g |
| 100 paternal half sibs | + | | + | | + | | + | | + | | + | |
| 50 paternal half sibs | | + | | + | | + | | + | | + | | + |
| Dam | + | + | + | + | + | + | + | + | + | + | + | + |
| Daughter | + | | | | | | | | | | | |
| Selected females (in %) | 77 | | 55 | | 45 | | 45 | | 35 | | 35 | |
| Generation interval | 4.25 yr | | 3.1 yr | | 2 yr | | 2 yr | | 1.75 yr | | 1.75 yr | |

considering an age at first calving of 25 mo and a calving interval of 13 mo. Because of the application of reproductive biotechnologies (embryo transfer and semen sexing), selection intensity also varied (due to the changing number of selection candidates).

The overall breeding goal of the dairy cattle farmer included one moderate heritability production trait (**MY**) and one low heritability functional trait (**FL**) with equal economic weights per genetic SD. According to selection strategies in dairy cattle farms, MY represents lactation milk yield from first parity, whereas FL was defined as functional longevity (in days). In total, seven on-farm breeding strategies for the cow-dam pathway of selection were evaluated. *Scenario I* reflects the conventional and conservative selection strategy, i.e., basing cow-dam selection on a cow's own performance. Hence, the cow dam selection pool only comprised lactating cows, implying a generation interval of 4.25 yr. In *scenario II*, selection is based on a female calf's genotype combined with phenotypic information from related animals. Selection at an early stage enlarges the pool of female selection candidates and implies a short generation interval of 3.1 yr. In *scenario III*, 50% of female heifers with highest genetic merit (according to pedigree index) were inseminated with sexed semen. Utilization of sexed semen increased selection intensity, due to fewer cows being required to generate the same number of female offspring. Generation interval was even lower than in *scenario II* and identical with the age at first calving because always a first calf of a selected heifer is female. *Scenario IV* was identical compared with s*cenario III* but used genomic estimated breeding values (**GEBV**) for pre-selection of heifers instead of pedigree indices. Reproduction technology ET was applied to 50% of genetically best heifers at the age of 12 mo according to pedigree index and according to GEBV in *scenario V* and *scenario VI*, respectively. Two female offspring per donor heifer and year, and a further decrease in generation intervals (1.75 yr), were assumed. An overview of index sources as considered in different scenarios is given in Table 1. Genetic parameters were obtained from a current study using test herds from the eastern part of Germany: $h^2 = 0.30$ for MY, $h^2 = 0.10$ for FL, and genetic and phenotypic correlations between MY and FL of 0.10 and -0.10, respectively. Phenotypic SD was 1,000 kg for MY and 500 d for FL.

Genomic index sources considered moderately accurate GEBV ($r_{MG} = 0.7$ for MY, and $r_{MG} = 0.5$ for FL). Highest genetic gain per year for MY and FL was identified for *scenarios III, IV, V, and VI* (Fig. 1). Compared with *scenarios I* and *II*, these scenarios strongly focused on the use of reproduction technologies. Hence, the decision to genotype heifers strongly depends on the assumptions for reproduction rates. König et al. (2007) analyzed ET traits such as the number of flushed and transferrable oocytes, and they found a substantial variation across herds and donor stations, and also a moderate genetic component. In a simulation study, Sorensen and Sorensen (2010) compared genetic gain in genomic breeding programs when either assuming one or five offspring per donor. Obvious success of genomic multiple ovulation and embryo transfer (**MOET**) breeding programs over conventional, or over genomic breeding programs without ET, required more than one female offspring per donor cow. For identical "reproduction scenarios," genetic

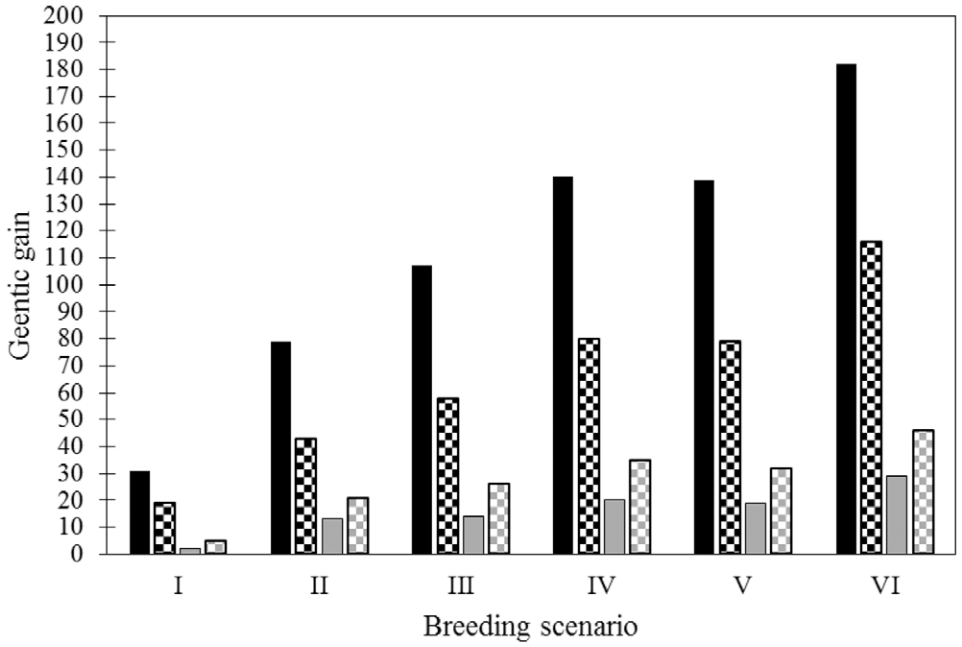


**Figure 1.** Genetic gain per year for milk yield (in kg) and functional longevity (in days). Black and gray bars = equal economic weights for milk yield and function longevity, respectively; black dotted and gray dotted bars = genetic gain for milk yield and functional longevity, respectively, when doubling the economic weight for functional longevity.

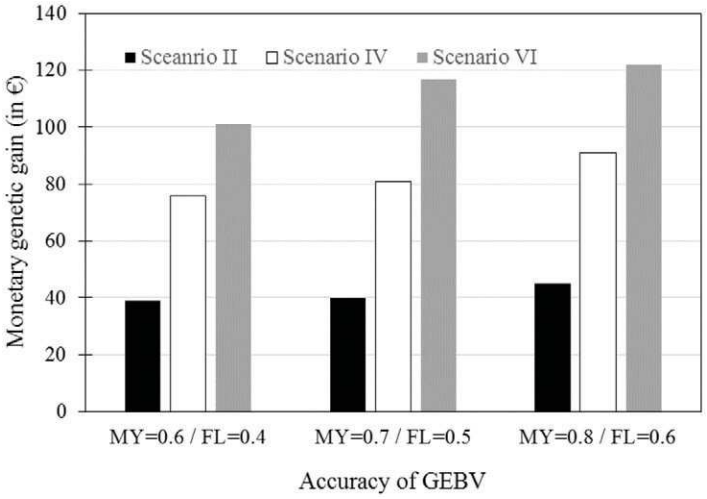


**Figure 2.** Additional monetary genetic gain of genomic breeding *scenarios II, IV, and VI* compared with the basic *scenario I*. Here: Equal economic weights per genetic SD for milk yield and functional longevity.

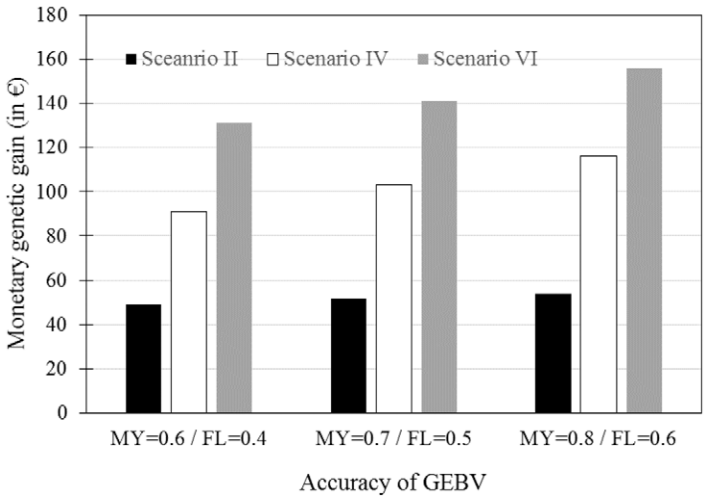


**Figure 3.** Additional monetary genetic gain of genomic breeding *scenarios II, IV, and VI* compared with the basic *scenario I*. Here: Economic weight per genetic SD for functional longevity is doubled.

gain was higher when basing selection of young heifers on GEBV instead on pure pedigree indices (comparison of *scenario IV* with *III*, and of *VI* with *V*). Increase of genetic gain in genomic scenarios was always higher for MY compared with FL. This is mainly due to the assumed higher accuracy of GEBV for MY. Also for identical accuracies, genetic gain was larger for the production trait, supporting results from deterministic economic evaluations on a long-term population-wide scale (König et al., 2009). In a combined breeding goal, more selection pressure is always on the higher heritability trait, and competiveness of low heritable functional traits is only possible when increasing economic weights for such traits. Similar genetic gain for the functional trait was also realized in the current investigation when doubling the economic weight for FL (Fig. 1, comparison of dotted bars).

Figure 2 displays additional annual monetary genetic gain (in euros) of genomic scenarios compared with *scenario I* for the combined breeding goal including MY and FL with equal economic weights per genetic SD. With regard to *scenario II*, for all accuracies of genomic predictions, and assuming genotyping costs of 50 euros/animal with the LD chip, selection of female calves based on GEBV did not compensate the costs for genotyping. When doubling the economic weight for FL (Fig. 3), additional monetary genetic gain of 55 euros compensated for the costs of genotyping, pre-assuming moderate accuracies of GEBV (0.70 for MY and 0.50 for FL). For high accuracies of GEBV (0.80 for MY and 0.60 for FL), additional monetary genetic gain was 61 euros.

Pryce and Hayes (2011) assumed genotyping costs of AU$29/animal, but discounted profit was AU$46/cow when genotyping 40 heifers to identify the top 20 as replacements for 100 cows. In their simulation study, Weigel et al. (2012) studied gains in lifetime net merit breeding values of selected females in commercial herds due to genomic testing by taking costs of genotyping into account (US$40 for the 3K low-density application). Economic gains increased with increasing selection intensity and with increasing incompleteness of pedigree information and were US$121 per female calf for a low within-herd replacement rate of 10%. As pointed out by Wiggans et al. (2011), availability of low-priced 3K SNP chips will also encourage commercial farmers to participate in genomic activities. Weigel et al. (2012) considered genotyping of a large number of females as a "significant financial investment." González-Recio et al. (2014a) estimated genetic (co)variances for feed efficiency traits using 843 genotyped heifers (632,000 SNPs). Estimates were used to incorporate residual feed intake of heifers into the Australian Profit Ranking index. Utilization of this index increased annual farm profitability by 3%, which implies an economic gain of AU$0.55 million considering the total Australian Holstein population.

## Optimization of mating designs

Application of an optimum genetic contribution (**OGC**) concept accumulates genetic gain by constraining inbreeding or additive-genetic relationships in a long-term perspective (e.g., Meuwissen, 1997). In this regard, and for a practical and sustainable implementation in conventional progeny-testing programs, König and Simianer (2006) recommended optimizing elite matings between bull dams and bull sires. In the genomic era and with a strong focus on improvements of functional traits, Schierenbeck et al. (2011) used semi-definite programming and relationships constructed from SNP data to define optimum genetic contributions for genotyped bull dams and bull sires. For a substantial minimization of inbreeding coefficients in the short term in the next progeny generation, Sonesson and Meuwissen (2000) developed a simulated annealing algorithm for the specification of mating designs using OGC output. Widespread genotyping of "commercial" cows additionally allows the optimization and specification of within-herd matings, also from the perspective of within-breed biodiversity. In his keynote about genomic breeding programs, Schaeffer (2006) suggested the calculation of heterozygosity indices based on SNP data for each genotyped animal and maximization of this index in the ongoing generation via specific mating designs.

Consideration of genomic information in mating designs allows exclusion of lethal recessives in the homozygous form. VanRaden et al. (2011) used SNP data to form haplotypes along the chromosome and compared observed and expected haplotype frequencies. They identified detrimental haplotypes with significant effects on paternal fertility and stillbirth. Based on the findings by VanRaden et al. (2011) for several cattle breeds, or by Kadri et al. (2014) for Nordic Red cattle, Swalve (2014) explicitly recommended inclusion of genomic data into mating plan software on a herd-gate level. Identification of individual functional mutations affecting male subfertility in the Fleckvieh population based on a genome-wide as-


sociation study (Pausch et al., 2014) strongly supports such enhancements of electronically organized mating plans.

As a further aspect, mating schemes including genomic data could exploit the effects of dominance. In a study by Varona et al. (1998), the dominance component explained 9% of the phenotypic variation for stature of dairy cattle. Consideration of dominance effects reflecting specific combining abilities allows for allocating the best sire for a given cow (DeStefano and Hoeschele, 1992). However, inclusion of dominance in traditional genetic evaluations is associated with increasing computational requirements (Miztal et al., 1998). In the genomic era with the availability of SNP data, Toro and Varona (2010) pointed to the necessity to re-evaluate models or selection strategies including dominance. In their simulation study for a random mating scheme, extra selection response for a whole-genome evaluation from an additive model additionally including dominance ranged from 9 to 14%. Even higher genetic gain was achieved when combining the dominance model with OGC theory. In a recent study, Ertl et al. (2014) used 777,962 SNPs from 1996 genotyped Fleckvieh cows. The proportion of genomic variance due to dominance was 3 to 50% of the genetic variance. In consequence, the authors saw the potential to consider dominance for planned matings, also being a motivation for commercial dairy cattle farmers to genotype their cows.

There is an increasing trend in dairy cattle farming worldwide to improve functional traits via crossbreeding. Freyer et al. (2008) reviewed and evaluated "crossbreeding experiments," but practical results partly lagged behind theoretical expectations. Exploitation of dominance based on high-density SNP marker data also might help to improve crossbreeding designs, i.e., for an accurate differentiation of breeds and genetics lines and for an accurate assessment of heterosis effects (Swalve, 2014). Identification of detrimental haplotypes or optimized mating schemes via consideration of dominance requires dense marker maps, but for commercial applications, farmers usually genotype female calves and heifers using low-density SNP chip panels. Nevertheless, efficient imputing strategies have been developed, either based on LD, family information, or a combination of both (Pimentel et al., 2013).

## Prediction of Phenotypes

### *Individual phenotypes in calibration groups*

A so called "genomic production value," defined as the sum of estimates for additive-genetic and dominance effects, might be an efficient selection instrument to optimize herd replacements and mating allocations. Estimation of both effects simultaneously requires the implementation of a cow calibration group, and using a cow's phenotype or corrected phenotype instead of a sire's EBV as dependent variable for the estimation of SNP effects. Compared with intensively pre-selected bulls as currently used in sire calibration groups, unselected cows represent genomic architecture of the broad milking cow population. Basing genomic selection on genotyped cows from the broad population might be associated with unbiased estimated SNP effects and unbiased genomic breeding values (Patry and Ducrocq, 2009).

Furthermore, cow calibration groups are imperative when focusing on novel traits that are not yet considered in official genetic evaluations. Most new traits of interest are strongly associated with animal robustness and require phenotyping strategies for the following trait categories health, workability, persistency, fertility, fitness, and mobility (Calus et al., 2013a). Nevertheless, Calus et al. (2013b) pointed to the generally antagonistic genetic relationships between new and conventional breeding goal traits and also discussed the generally small economic weights for new traits. Both components, antagonistic associations and small economic values, require reference populations including at least 10,000 individuals (Calus et al., 2013b). Otherwise, selection response for new traits will be extremely small.

Accuracy of derived SNP effects or genomic breeding values from reference groups basing on individual phenotypes strongly depend on the group size, on the heritability of the trait, and on genomic architecture reflected by LD or effective population size (Goddard, 2009). Following Goddard's deterministic prediction equation, more than 20,000 cows need to be genotyped to realize moderate accuracies of genomic predictions larger than 0.5 for a low heritability trait ($h^2 \sim 0.05$). However, without availability of highly accurate conventional sire EBV, a crucial question remains the validation of estimates from cow calibration groups for novel traits. In a first "German cow calibration study," Becker-Scalez et al. (2015) based their studies on a subset of 3,521 genotyped Holstein cows. With a focus on longitudinal test-day milk yield, random regression coefficients (**RRC**) from Legendre polynomials of order 2 were defined as phenotypes in the reference set to predict genomic random regression coefficients for cows in the validation set. A fivefold cross validation was done to evaluate the accuracies of genomic intercepts, linear and quadratic RRC (**gRRC0**, **gRRC1**, and **gRRC2**, respectively). For this purpose, the whole dataset of genotyped cows was randomly divided into five groups, and cows' gRRC of one group were assumed to be unknown (validation

set). Effects of SNP estimated from the other four groups were used to predict the gRRC of cows in the validation set. Cross validation was repeated five times for each coefficient. Pearson's correlation coefficients between the realized RRC and the predicted gRRC for the animals in the validation set were calculated for each coefficient in each replicate. Moderate correlations were found between RRC and predicted gRRC, i.e., 0.62 ± 0.02 for RRC0, 0.67 ± 0.01 for RRC1, and 0.69 ± 0.02 (Fig. 4). Utilization of cow prediction and validation sets exhibiting substantial differences or obvious similarities regarding disease incidences, production levels, genomic architectures, and genetic relationships underlines the necessity of complex cross-validation studies. Pérez-Cabal et al. (2012) presented and compared a variety of cross-validation strategies, also for within or across generation predictions.

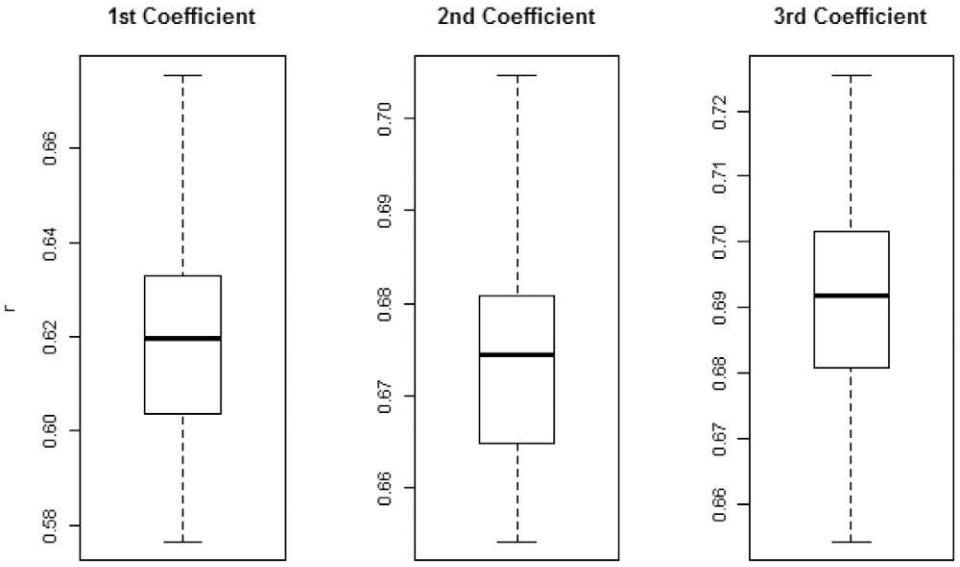


**Figure 4.** Box plot for the correlations between realized random regression coefficients and predicted genomic random regression coefficients for the cows in the validation set (according to Becker-Scalez et al., 2015).

The advantages and flexibilities of "random regression phenotypes" for genetic evaluations were clearly outlined by Santos et al. (2010). Silva et al. (2013) used parameter estimates from nonlinear logistic regressions as phenotypes for the modeling of genomic growth curves in pigs. Also in this study, independence of growth curve modeling and genomic modeling simplifies statistical procedures and reflects a classical two-step procedure for longitudinal data, i.e., basing genetic marker predictions on pre-corrected phenotypes (Pong-Wong and Hadjipavlou, 2010).

A detailed overview of non-parametric machine-learning procedures for genome-assisted predictions is given by González-Recio et al. (2014b). Generally, a machine-learning technique uses past events to set up a prediction model for the interpretation of new information and is suitable to process invisible information from large datasets, especially from datasets characterized by a large number of markers in relation to a small number of genotyped animals. Random forest (**RF**) is one of the suggested specific machine-learning methods. In a further cow calibration study, Naderi et al. (2014) used simulated binary and real binary data from genotyped cows to predict phenotypes for disease traits based on RF methodology (e.g., González-Recio and Forni, 2011). In the stochastic simulation, 20% of phenotyped females from the last two generation were defined as sick and received the code 1, and the remaining healthy cows received the code 0. Females from the last two generations were divided into a reference and a validation set. Phenotypes of the animals in the validation set were assumed to be unknown. In different scenarios, reference and validation sets were created according to the health status of cows. In the basic scenario, the total number of sick cows (4,000 cows) was assigned to the validation set, and 16,000 healthy cows were assigned to the reference set. Main evaluation criterion was the area under the receiving operating characteristic curve (**AUC**). The AUC reflects results for the comparisons of true positive, false positive, true negative, or false negative outcomes with a predicted disease based on genomic information from related animals. An AUC close to 1 indicates an accurate predictive ability (González-Recio et al., 2014b), which was higher throughout our simulation study for RF compared with GBLUP for all variants of cow allocations to either reference or validation sets (Table 2). Lowest prediction accuracies were found for a low percentage of sick animals in the reference set. Using real phenotypes for clinical mastitis from 6,762 genotyped cows, calculated AUC values ranged from 0.53 to 0.57 for a variety of training and validation set compositions (variations due to group sizes and due to the percentage of sick animals in both groups). Again, highest AUC were identified when disease incidences in calibration groups reflected population disease incidenc-

**Table 2. Area under the receiving operating characteristic curve (AUC) for the prediction of phenotypes of disease traits in the validation set for GBLUP and random forest (RF) applications (50K SNP chip, $h^2$ = 0.10 and 725 QTL). Values in parenthesis show the SDs from 10 replicates (according to Naderi et al., 2014).**

| | Percentage and number of sick animals in reference set[1] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 10% 400[2] | 20% 800[2] | 30% 1200[2] | 40% 1600[2] | 50% 2000[2] | 60% 2400[2] | 70% 2800[2] | 80% 3200[2] | 90% 3600[2] | 100% 4000[2] |
| RF | 0.57 | 0.598 | 0.60 | 0.59 | 0.60 | 0.61 | 0.63 | 0.63 | 0.64 | 0.63 |
| | (0.06) | (0.03) | (0.04) | (0.03) | (0.03) | (0.04) | (0.03) | (0.04) | (0.04) | (0.04) |
| GBLUP | 0.58 | 0.64 | 0.64 | 0.66 | 0.66 | 0.63 | 0.64 | 0.66 | 0.64 | 0.63 |
| | (0.01) | (0.02) | (0.02) | (0.02) | (0.03) | (0.04) | (0.02) | (0.01) | (0.01) | (0.03) |

[1]Size of reference and validation included 16,000 and 4,000 cows, respectively, for all scenarios.

[2]Number of sick animals assigned to the reference set (number of healthy animals in reference set is the difference between this number and the total number of cows ($N$ = 16,000) in the reference set).

es. Luan et al. (2009) used a sire calibration group, GBLUP, and Bayesian methods to estimate genomic breeding values for clinical mastitis in Norwegian Red cattle. They identified variations in accuracies of genomic predictions for clinical mastitis records from different lactation stages, probably due to different heritabilities, and also due to variations of disease incidences.

In a key publication addressing phenotype predictions based on genomic data, Ober et al. (2012) used ~2.5 million SNP from whole-genome sequence data to predict phenotypes for starvation stress resistance and locomotor startle response in *Drosophila melanogaster*. Statistical methodologies included GBLUP and Bayes B with internal SNP selection. Predictive ability was defined as the correlation between genomic breeding values and phenotypes for *Drosophila melanogaster* in the validation set based on a series of fivefold cross validations. Ober et al. (2012) only found a moderate predictive ability, i.e., 0.239 ± 0.008 for starvation resistance and 0.2330 ± 0.012 for locomotion startle response, and also only minor differences for GBLUP and Bayes B applications. A crucial threshold of 150,000 SNPs was identified, implying a minor increase of predictive ability with increasing numbers of SNPs above this threshold. The authors partly attributed the moderate predictive ability to the sample size of 157 lines, but they also defined the potential of quantitative trait predictions based on genome information, especially for evolutionary biology and medicine. Hence, a first simulation study for phenotype prediction based on dense genomic marker data focused on the prediction of individual genetic risk to a specific disease in case-control studies (Wray et al., 2007). Ongoing research is aimed at a mouse population and the prediction of unobserved phenotypes for coat color and mean cell hemoglobin using high-density SNP data (Lee et al., 2008). Unobserved phenotypes were predicted using a model including simultaneously genomic additive and genomic dominance effects. Using a family design (splitting families into an estimation and into a prediction group with equal frequencies), correlations between predicted and real phenotypes (= prediction accuracies) were in a moderate to high range from 0.4 to 0.9.

### *Prediction of genotypes and phenotypes adopted to environments*

In plant breeding, multi-environment models have been developed for genomic predictions of breeding values and for performance prediction of untested genotypes by considering genotype × environment interactions (Burgueño et al., 2012). Their multi-environment model considered specific within-line across-environment covariance structures based on genomic or pedigree relationships or by combining both genomic and pedigree information. Performance prediction of genotypes with genetic evaluations in some specific conventional environments, but not in, e.g., harsh environments, also addresses practical animal breeding. Increasing heat stress especially depresses productivity, reproduction, and health of dairy cattle kept in outdoor systems. The most used continuous environmental descriptor reflecting heat stress in farm animals by combining air temperature with humidity is the temperature-humidity index (**THI**) (e.g., Bohmanova et al., 2007). In their stochastic simulation study, Yin et al. (2014) used genomic random regression models (**gRRM**) to predict cattle performances in dependency of THI, assuming a variety of genomic architectures. Repeated measurement analysis revealed highly accurate predictions for cows with SNP genotypes but without phenotypes in defined harsh environments, e.g., for THI 75. However, according to their findings, a small fraction of 20% of animals needs to be phenotyped for the trait of interest at THI 75. Consideration of the environmental impact and of possible genotype by environment interactions in genomic predictions is of practical relevance. Examples address the background of livestock and semen exports in tropical or subtropical countries or the transfer of SNP prediction equations from conventional to organic production systems.

Grazing heifers from the research station of Kassel university: Genotyping as a tool for phenotype prediction (source: © 2015 König).

Phenotypic modifications for an identical genetic background due to environmental changes were described by Gause (1947), who introduced the term "phenotypic plasticity." In a present study based on genomic data from the German cow calibration group, phenotypic plasticity and variation of genetic parameters were studied by consideration of both environmental variation and genetic architecture effects. Considering both effects simultaneously, this approach will also evaluate Robertson's (1959) recommendation to use a genetic correlation of 0.80 or lower as general indicator for genotype by environment interactions. For the two strata herd size (environmental effect) and average herd LD measured as $r^2$ between all possible SNP pairs (herd characteristic on the genomic scale), and using 6,616 genotyped cows (50K), the largest additive-genetic variances were identified for large within-herd $r^2$ in large-scale herds. Genetic correlations in the same traits across herds were lower than 0.80 for substantial differences in genetic herd architectures (e.g., level of inbreeding, level of LD, and percentage of daughters from influential sires from North America).

## Conclusion

Farmers are encouraged to genotype female calves and heifers to accelerate genetic gain on the cow-dam pathway of selection. Detection of the most promising heifers for replacements, and the specification of mating schemes, are valuable instruments to improve the overall farm management. Inclusion of genomic information into selection and mating instruments substantially improves accuracy of selection compared with the utilization of "conventional" pedigree indices. Exploration of non-additive genetic effects based on SNP data can be used to optimize

specific matings within breeds as well as for the selection of optimal lines in crossbreeding designs. A further farm management component from an animal breeding perspective addresses controlling of inbreeding and genetic relationships in a long-term perspective. Heterozygosity indices based on SNP data and avoiding lethal recessive genotypes might have practical applications in the near future. A variety of novel traits reflecting resource efficiency (e.g., methane emissions) or product quality (e.g., milk fatty acid composition based on spectral data), while also reflecting the demand of consumers, might be interesting for modern breeding goals. However, without availability of highly accurate conventional sire EBV, it is imperative to implement cow calibration groups that are directly based on cow phenotypes. Using cow calibration groups for the derivation and validation of SNP effects requires alternative statistical modeling, such as random forest methodology, or extensions of random regression models for longitudinal data. Further potential of genomic random regression models is related to a variety of specific breeding scenarios, e.g., the prediction of genomic breeding values for animals without phenotypes in harsh environments.

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## About the Authors

**Sven König** is Professor of Animal Breeding at the University of Kassel, Germany. He studied animal breeding at the University of Göttingen, and he was a visiting researcher at the University of Guelph. His main research interests include population genomics, quantitative genetics, and evaluation of breeding program designs in farm animals, especially in dairy cattle. A strong focus of his research aims at the genetic improvement of functional traits by applying genomic selection strategies. One recent collaborative project, "cow calibration groups," is based on 20,000 genotyped cows with phenotypes for a variety of disease traits, product quality, as well as behavior and temperament traits. Development of genetic-statistical modeling also considers a strong environmental component, e.g., heat stress indicators, for further explanations of genotype by environment interactions on genomic and quantitative-genetic scales. He has published more than 70 peer-reviewed scientific full-length papers, and he currently supervises 13 Ph.D. and 10 master's students. Since 2015, he is vice-president of the EAAP Cattle Commission.
**Correspondance:** sven.koenig@uni-kassel.de

**Tong Yin** is Senior Researcher in the animal breeding group at University of Kassel, Germany. She received her master's degree at Shandong Agricultural University in China. In the framework of her master program, Dr. Yin compared and evaluated a variety of QTL mapping strategies. Afterwards, she was Ph.D. student in the animal breeding and genetics group at the University of Göttingen. During her Ph.D. study, she was involved in a collaborative EU project aiming on the implementation of genomic selection for dairy cattle in low-input production systems. Currently, she is project partner of the German "cow calibration group" project. The overall goal of this project is to implement genomic selection in German Holstein dairy cattle for novel traits based on 20,000 genotyped cows. Specifically, Dr. Yin studies aspects of genotype by environment interactions by combining continuous environmental descriptors and measurements for genomic architectures. Her further research interests focus on enhancements of statistical modeling using genomic data. Dr. Yin is also involved in international teaching programs, and she supervises master's and Ph.D. students in population genomics topics.
**Correspondance:** tong.yin@uni-kassel.de

# 3. General discussion

## 3.1. Genetic architecture of functional traits

In general, most of the functional traits in dairy cattle (e.g., health disorders, fertility, and behavior) are controlled by numerous genes with minor effects, implying only low heritabilities. However, a few examples for functional traits with moderate to high heritabilities exist, e.g., body weight and feed efficiency (Byskov et al., 2017), and resistance to specific viral and bacterial pathogens (Mahmoud et al., 2018). Furthermore, for diseases within the same trait category, the genetic background varied substantially (Mahmoud et al., 2018). In the ongoing studies and overall discussion of this thesis, focus is on genetic architecture analyses for functional traits as presented in **original research paper 3** to **7**, i.e., methane emissions, body weight and behavior traits.

### 3.1.1. Methane

Heritabilities for CH4 differed considerably, depending on prediction equations, measuring approaches and population characteristics. For example, the average heritabilities across first lactation from the two different prediction equations were 0.25 and 0.31 (**original research paper 3**). An even broader CH4 heritability range for CH4 was presented by van Engelen et al. (2015). They predicted CH4 based on milk fatty acids (Dijkstra et al., 2011) recorded in 1,905 first-lactation Dutch Holstein-Friesian cows, and they used three different prediction equations. The CH4 heritabilities were in the range from 0.05 to 0.30. A few years later, the same authors (van Engelen et al., 2018)reported a heritability of 0.11 for CH4, based on measurements from infrared sensors installed in automatic milking systems. Ricci et al. (2014) measured CH4 with a portable handheld laser methane detector, and classified the overall CH4 emission into respiration CH4 and eructation CH4. In a CH4 study including 330 ewes, Reintke et al. (2020) followed the recording protocol by Ricci et al. (2014), but estimated CH4 heritabilities for different respiration and eructation traits were very small, with a maximal heritability of 0.04.

Pinto et al. (2020a) recorded CH4 emissions in exotic, indigenous and crossbred cows in India using the portable hand-held laser methane detector. The variance explained by the simple random cow effect in relation to the total variance ranged from 0 for the CH4 mean to 0.10 for maximal respiration CH4 within a measuring duration of 2 minutes. The small CH4 cow

variance as measured in the harsh environment in India along heterogeneous social-ecological gradients indicates a small genetic variance component. However, most of the genetic CH4 studies conducted in cattle or in small ruminants base on a quite small number of phenotyped animals. In consequence, all available data sources for direct and indirect CH4 measurements, also from an across-country perspective, should be considered for CH4 genetic evaluations. Against this background, de Haas et al. (2017) suggested residual feed intake and milk fatty acid composition as proper CH4 indicator traits, and they suggested international cow reference populations for genomic predictions.

Genome-wide association studies for direct CH4 and adjusted CH4 (i.e., adjustments through correlated traits) reflect the polygenic nature of the trait. For example, Pszczola et al. (2018) converted concentrations of CH4 and CO2 in 287 Polish Holstein-Friesian cows, which were measured by infrared sensors during milking in automatic milking systems, to daily CH4. They identified 50 significantly associated SNP, which only explained 0.154% of the total genetic CH4 variation. Calderón-Chagoya et al. (2019) applied multi-breed GWAS using daily CH4 concentrations from 280 cows. Again, a similar number of 46 significant SNP with small effects, were detected. The significant SNP reported from both studies are located in genomic regions with impact on milk compositions and feed efficiency, such as fatty acid compositions, daily gain, live weight and residual feed intake. Hence, shared biological pathways for CH4, milk fatty acids and feed efficiency, are assumed. However, when using both direct CH4 and adjusted CH4 as phenotypes in GWAS (i.e., correction of CH4 emissions on dry matter intake and live weight), only a scarce number of shared significant SNP was detected (Manzanilla-Pech et al., 2016). The explanation was the small phenotypic and genetic correlation between adjusted and direct CH4. In other words, adjusted CH4 and raw CH4 are different traits. Genetic variances and heritabilities for CH4 emissions slightly increased when the **G** matrix was built based on approximately 3,300 significant SNP for dry matter intake and live weight (Manzanilla-Pech et al., 2016). Such result indicates that genetic CH4 variation is largely captured by SNP contributing to strongly correlated feed efficiency traits.

### 3.1.2. Body weight

Previous studies (Coffey et al., 2006; Lassen and Løvendahl, 2016) and the results from the **original research papers 4** to **6** indicate that BW is a moderate to high heritability trait. However, the proportion of genetic variations depends on populations and on age stages.

Coffey et al. (2006) and Brotherstone et al. (2007) estimated heritabilities slightly larger than 0.50 for BW at birth, and of 0.75 and 0.59, respectively, for BW at the first calving date. Similarly, quite large heritabilities based on a longitudinal monthly data structure are presented in the **original research paper 4**. Furthermore, in this study, maternal genetic and maternal permanent environmental effects for BW were included in RRM. The maternal genetic effect accounted for 18% of the total BW variance at birth, but substantially decreased (as expected) with aging. The gradually decrease in maternal heritabilities from birth to age month 5, and the extremely low maternal heritabilities in later life, indicate the importance of the maternal environment in dairy cattle only close to the calving date. Variances for the maternal permanent environmental effects were only 0.40 $kg^2$ and 0.65 $kg^2$, respectively (as presented in the **original research papers 4** and **6**). However, on average 1.22 phenotyped daughters per dam might not be sufficient to partition properly the maternal component into the maternal permanent environmental variance component and the maternal genetic variance component.

Interestingly, moderate to high heritabilities were also reported for growth curve parameters from different nonlinear functions, irrespective of the applied relationship matrices, i.e., the pedigree-based relationship matrix (**A**) as used by Meyer (1995) and Forni et al. (2007), or the genomic relationship matrix (**G**) and the combined relationship matrix (**H**) as considered in the **original research paper 6**. In our study, we additionally modelled nonlinear functions with curve parameters reflecting biological meanings. Specifically, apart from the shape parameter from the Richards function, the other three curve parameters reflect birth weight, mature weight and growth rate. In consequence, the moderate to high heritability estimates for the curve parameters are in line with heritabilities for birth weight, mature weight and growth rate.

Only a limited number of SNP was significantly associated with birth weight, weaning weight and insemination weight (as shown in the **original research paper 5**). Nevertheless, because of the heritabilities for BW which were larger than 0.36 when using the **A** matrix, stronger SNP effects were expected. In addition, the very similar and small genetic variances for BW on chromosome levels indicate the polygenic inheritance of BW. As suggested by Manzanilla-Pech et al. (2016), only SNP with *P-values* < 0.05 for one BW trait were kept to estimate the variance components and heritabilities for the other two BW traits. Compared to the "full matrix" approach using all available SNP, the genetic variances for the BW trait of interest decreased, when only the significantly associated SNP for the two other BW traits were used to build the **G** matrices (Table 1). Consequently, except for BW recorded at 2 to 3 months of age (**BW23**), heritabilities based on the SNP subsets were substantially smaller (reduction up

to factor 0.5) compared to the heritabilities when using all SNP. Such results indicate, apart from BW23, that ~2,000 significant SNP (*P-values* < 0.05) explain half of the genetic variation, which is captured by ~42,000 SNP (Table 1). For BW23, ~2,000 SNP explain as much as genetic variance as the underlying genes that link to ~42,000 SNP across the genome. Hence, as indicated in Table 1, BW from different ages along the growth trajectory have a changing genetic background, i.e., different SNP (and consequently linked genes) contribute to trait responses. In this regard, Zhang et al. (2014) suggested to consider SNP with specific weights to build trait-specific **G** matrices, in order to improve the accuracies of genomic predictions. However, comparing to the construction of the **G** matrix as suggested by vanRaden (2008), tremendous efforts are required, i.e., to collect prior information for each SNP and each trait. Moreover, only minor improvements are expected when all underlying functional genes similarly contribute to the phenotype of interest.

**Table 1.** Genetic ($\sigma_a^2$), residual ($\sigma_e^2$), phenotypic variances ($\sigma_p^2$), heritabilities ($h^2$), and standard errors of heritabilities (SE) for body weight recorded at birth (BW0), at 2 to 3 months of age (BW23) and at 13 to 14 months of age (BW1314) estimated via models using all SNP (ALL) and models only considering SNP with *P-values* < 0.05 for BW0 (sBW0), BW23 (sBW23), and BW1314 (sBW1314).

| Trait | SNP | #SNP | Genetic parameter | | | | |
|---|---|---|---|---|---|---|---|
| | | | $\sigma_a^2$ | $\sigma_e^2$ | $\sigma_p^2$ | $h^2$ | SE |
| BW0 | ALL | 42,468 | 6.08 | 12.37 | 18.46 | 0.33 | 0.01 |
| | sBW23 | 2,071 | 3.08 | 14.99 | 18.06 | 0.17 | 0.01 |
| | sBW1314 | 2,083 | 3.14 | 14.96 | 18.11 | 0.17 | 0.01 |
| BW23 | ALL | 42,388 | 20.28 | 84.54 | 104.82 | 0.19 | 0.02 |
| | sBW0 | 2,087 | 14.80 | 89.35 | 104.18 | 0.14 | 0.02 |
| | sBW1314 | 2,083 | 15.05 | 88.43 | 103.48 | 0.15 | 0.02 |
| BW1314 | ALL | 42,443 | 174.96 | 604.65 | 779.61 | 0.22 | 0.02 |
| | sBW0 | 2,087 | 95.56 | 669.54 | 765.10 | 0.13 | 0.01 |
| | sBW23 | 2,071 | 89.40 | 674.69 | 764.08 | 0.12 | 0.01 |

### 3.1.3. Behavior traits

According to the genetic variances and heritabilities as reported in **original research paper 7**, natural bovine behavior of cows including feeding (**FEED**), ruminating (**RUM**), resting / non-active (**NACT**), basic activity (**BACT**) and high activity (**HACT**), is under low to moderate genetic control. Among the behavior traits recorded via sensor technology, FEED, RUM, and NACT displayed larger heritabilities than RUM and BACT. A small number of significant SNP were detected for RUM, FEED and NACT, when considering the very relaxed false discovery rate of 20%. With regard to the stringent Bonferroni threshold, only one SNP was significantly associated with NACT. Hence, the genetic and genomic studies for automatically recorded behavior via sensor technology confirms that basic cattle habits are controlled by numerous loci with mainly small effects.

**Table 2.** Genetic ($\sigma^2_a$), permanent environmental ($\sigma^2_p$), residual variances ($\sigma^2_e$), heritabilities ($h^2$), and standard error of heritabilities (SE) for behavior traits using the combined pedigree (**A**) and genomic (**G**) relationship matrix with different numbers of SNP.

| | Combined **A** and **G** with 35,826 SNP | | | | | Combined **A** and **G** with 1,011 SNP[1] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Trait | $\sigma^2_a$ | $\sigma^2_{pe}$ | $\sigma^2_e$ | $h^2$ | SE | $\sigma^2_a$ | $\sigma^2_{pe}$ | $\sigma^2_e$ | $h^2$ | SE |
| RUM | 0.84 | 10.52 | 29.59 | 0.02 | 0.05 | 0.49 | 10.82 | 29.59 | 0.01 | 0.03 |
| FEED | 9.51 | 3.63 | 35.48 | 0.20 | 0.05 | 5.91 | 6.77 | 35.48 | 0.12 | 0.04 |
| BACT | 1.57 | 5.04 | 18.47 | 0.06 | 0.05 | 0.51 | 5.96 | 18.47 | 0.02 | 0.03 |
| HACT | 1.68 | 0.72 | 6.01 | 0.20 | 0.05 | 1.08 | 1.26 | 6.01 | 0.13 | 0.04 |
| NACT | 7.79 | 6.69 | 28.60 | 0.18 | 0.06 | 6.48 | 7.78 | 28.60 | 0.15 | 0.05 |
| WEL_IP | 0.08 | 0.19 | 1.88 | 0.04 | 0.02 | 0.05 | 0.21 | 1.88 | 0.02 | 0.02 |
| WEL_IC[2] | 0.07 | 0.10 | 1.56 | 0.04 | 0.02 | 0.04 | 0.13 | 1.56 | 0.02 | 0.01 |

RUM = rumination; FEED = feeding; BACT = basic active; HACT = high active; NACT = not active; WEL_IP = welfare index point; WEL_IC = welfare index class; [1]: SNP within a window of 50 kb up- and downstream of the 445 homologous genes; [2]: Genetic, permanent environmental, and residual variances for WEL_IC are multiplied with 10.

In a further approach, 1,011 SNP within a window of 50 kb up- and downstream of 445 homologous genes were considered to construct a reduced **G** matrix. The 445 genes were

involved in the biological process of behavior as mentioned in the **original research paper 7**. Using the reduced **G** matrix, genetic variances and heritabilities for all behavior traits decreased (Table 2). Interestingly, the decrease in genetic variation was associated with an increase of the permanent environmental variance component. Residual variances were almost identical when modelling the full or the reduced genomic relationship matrices. Among all behavior traits, NACT displayed the smallest heritability decrease, indicating that the 1,011 SNP near the 445 homologous genes are able to capture almost all the genetic variation, which is explained by the full number of 35,826 SNP. In other words, the 445 genes might comprise most of the genomic regions contributing to NACT. However, for the very low heritability traits (RUM, BACT, WEL_IP and WEL_IC), functions of the 445 genes were not conserved in the process of evolution.

### 3.2. Genotype by environment interaction

A comprehensive study to test G×E for production and health traits as well as for the length of productive life (**LPL**) considering conventional and organic production systems was conducted by Shabalina et al. (2019). Genetic correlations for disease traits and LPL between organic and conventional production systems using either the **A** or the **G** matrices were lower than 0.80, indicating obvious G×E. In contrast, genetic correlations between moderate heritability test-day production traits from the different systems were larger than 0.80. In an ongoing approach using the genomic marker data, patterns of genome-wide associations were different for LPL and health traits in both production systems, and different gene networks and causal pathways were identified. Generally, environmental sensitivity on quantitative-genetic and genomic levels was generally stronger for functional than for production traits. For continuous environmental descriptors, results from the **original research papers 1** and **2** indicated the influence of heat stress on simulated traits and production traits across THI levels, based on the **A**, **G** and **H** matrices. Additionally, results from the **original research paper 8** displayed alterations of estimated (co)variance components across continuous genomic, genetic and phenotypic herd descriptors.

#### 3.2.1. Discrete environmental descriptor: Conventional and organic production systems

As indicated above, Shabalina et al. (2019) estimated quite large genetic correlations between production traits from organic and conventional herds, e.g. of 0.85 (standard error (**SE**) of 0.03)

for lactation fat percentage. However, with regard to health traits, the genetic correlations decreased to 0.41 (SE of 0.21) for mastitis, 0.34 (SE of 0.18) for ovarian cycle disorders, and 0.30 (SE of 0.14) for digital dermatitis. The genetic correlation between LPL recorded in organic herds and in conventional herds was 0.67 (SE of 0.13). According to a threshold of $r_g \leq 0.80$ as suggested by Robertson (1959), considerable G×E existed for all functional traits. Compared to production traits, lower genetic correlations with larger SE were expected for health traits, because the multiple-trait modelling approach without residual covariances is a challenge for low heritability traits, especially for binary traits with low disease incidences (Calus et al., 2004). For milk yield, the genetic correlation was larger when modelling the **G** matrix compared to the modelling approach with the **A** matrix, because the **G** matrix reflects the similarity between animals based on SNP markers instead of common ancestors.

### 3.2.2. Temperature-humidity-index

In addition to the general approach using discrete environmental descriptors (as outlined above by Shabalina et al. (2019)), the present thesis focuses on the estimation of (co)variance in dependency of continuous explanatory variables such as THI. In contrast to the quite large genetic correlations among THI levels as reported by Brügemann et al. (2011), the genetic correlations for milk yield between low and high THI were 0.49 (based on the **A** matrix) and 0.18 (based on the **H** matrix), implying re-ranking of sires in genetic evaluations across THI. Interestingly, for somatic cell score, the corresponding genetic correlations were 0.85 for the **A** matrix and 0.80 for the **H** matrix. Somatic cell score seems to be a specific trait in genomic evaluations for German Holstein cows. In official genetic evaluations, prediction accuracies for low heritability somatic cell score (**SCS**) are larger than for the moderate heritability traits milk yield or fat percentage. Hence, traits with fundamental differences in genetic architectures, e.g., in the number of segregating QTL, in the distribution of QTL effects, and in variance ratios for additive genetic, dominance and epistatic effects, show specific (unexpected) covariances with respect to alterations of environmental gradients. In this regard, when using repeated test-day instead of lactation records, both between-cow and within-cow variations can be used to infer possible G×E in more detail (Hayes et al., 2003).

Another topic for differences in G×E studies addresses animal versus sire model applications. In the present thesis in **original research papers 1, 2, 3,** and **4**, RRM animal models were applied to analyze a longitudinal data structure. Especially in heat stress studies, animal models

might be more suitable, because the same cow has the possibility to produce under various climatic conditions. In consequence, a more accurate G×E prediction is expected from animal model than from sire model applications. The very similar modelling strategy in this thesis when compared to Hayes et al. (2003) displayed a very similar behavior of genetic (co)variance pattern for longitudinal test-day production traits in both studies. The general superiority of animal over sire model applications, e.g., the more detailed possibilities to estimate variance components, was described by Hudson and Schaeffer (1984). Ramirez-Valverde et al. (2001) and Sun et al. (2009) confirmed the animal model superiority via EBV evaluations, e.g., larger EBV accuracies and improved EBV stabilities.

### 3.2.3. Herd descriptors

Phenotypic herd descriptors (herd size and herd non-return rate) as well as novel genetic and genomic herd characteristics (intra-herd inbreeding coefficient, percentage of daughters from foreign sires, level of LD within specific chromosome segments, allele frequency for a SNP within the *DGAT1* gene) were continuous explanatory variables in the G×E study in the **original research paper 8**. A reference scenario addressed random herd descriptors. Again, a focus was on RRM applications. In addition, in further validations, multiple trait models were applied, implying the allocation of herds into different groups named “high”, “middle” and “low”. This was done in consecutive runs for all herd descriptors. For the herd descriptor based on random numbers, the assigning and analyzing procedure was repeated for 50 times, in order to generate the mean and standard deviation (**SD**) for all parameter estimates. The heritabilities for SCS were quite constant across the random descriptor scale (Figure 1). For production traits, heritabilities slightly increased at the curve peripheries. The genetic correlations curves were almost identical for milk composition traits and SCS, i.e., in the range from 1.00 to ~ 0.75, and from 1.00 to 0.62 for milk yield. With regard to the random herd allocation approach, genetic correlations between same traits form high, middle and low random descriptor classes were always larger than 0.81, indicating that G×E were non-existent. In contrast, obvious G×E were identified for milk yield for all herd descriptors (except for the allele frequency of the SNP located in the *DGAT1* gene), for fat percentage in dependency of the herd non-return rate and herd inbreeding coefficient, for protein percentage in dependency of herd size, and for SCS in response to herd size, herd non-return rate, LD and percentage of foreign sire alterations. Interestingly, the genetic correlations were larger than 0.62 when considering the extreme allele frequencies, but were substantially lower when correlating the herds with the best and the worst

non-return rate. Although the *K232A* substitution within *DGAT1* is in complete LD with the SNP *ARS-BFGL-NGS-4939* (Wang et al., 2012), the allele frequency of the SNP is not sufficient to fully represent the gene segregation. Further polymorphic variants were detected in the *DGAT1* gene (Kühn et al., 2004). Consequently, the single SNP within the *DGAT1* gene captures only a small proportion of the variance. In this regard, Fang et al. (2014) identified small heritabilities on a SNP basis with 0.06 for milk yield, 0.03 for fat yield and 0.02 for protein yield. Hence, due to the small genetic variation from single SNP, it is questionable to consider allele frequencies in comprehensive G×E studies. However, also non-return rate is a very low heritability fertility trait, displaying heritabilities of 0.03 and 0.02 in the **original research paper 4**, but in contrast to single SNP, intra-herd non-return rate also reflects environmental variations. The descriptor herd size comprises a broad pattern of environmental effects, due to associations with feeding, husbandry, and management conditions. Accordingly, considerable G×E were observed for milk yield and SCS when considering the largest and the smallest herds. Hence, variation in the herd environment contributed to genetic differentiation.

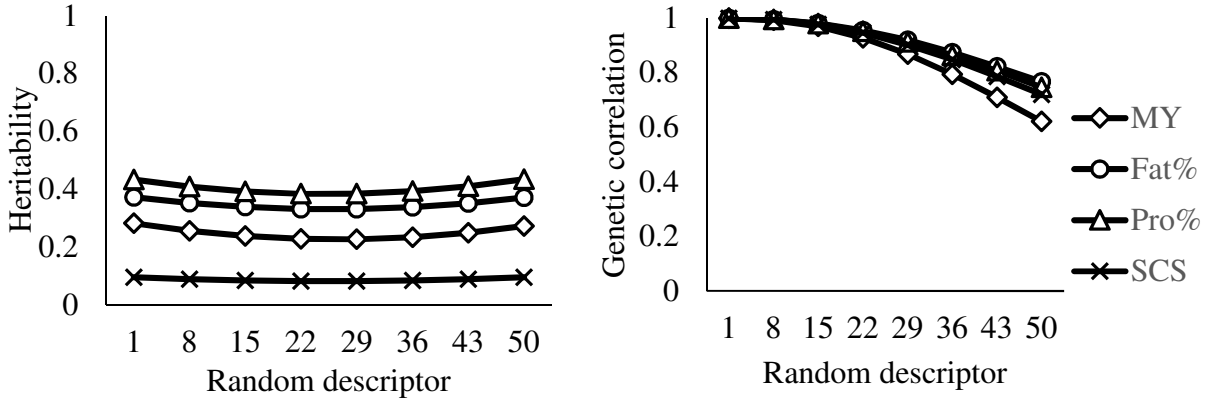


**Figure 1**. Heritabilities and genetic correlations between the minimal level and remaining levels for the random herd descriptor considering the following test-day traits: milk yield (MY), fat percentage (Fat%), protein percentage (Pro%), and somatic cell score (SCS). Standard deviations (SD) for posterior heritability estimates ranged from 0.005 to 0.044 for MY, from 0.005 to 0.042 for Fat%, from 0.006 to 0.045 for Pro%, and from 0.002 to 0.018 for SCS. SD for posterior estimates of genetic correlations ranged from 0 to 0.126 for MY, from 0 to 0.122 for fat%, from 0 to 0.099 for Pro%, and from 0 to 0.167 for SCS; SD for genetic correlations increased with increasing distance between herd descriptors.

### 3.2.4. Future environmental descriptors

The survey stratification index (Hoffmann et al., 2017), considering build-up density and the distance to the city center, was defined as a continuous environmental descriptor to reflect social-ecological characteristics in harsh environments in Bangalore in India (Pinto et al., 2020b). This index comprises not only the aforementioned environmental components, but also human-animal relationships and interactions. Results from linear mixed model applications displayed the variation in productivity and functionality along the rural-urban interface (Pinto et al., 2020b). In particular, compared to rural strata, cows kept in urban farms responded with higher milk yield and desired body condition and hygiene scores. The cows in urban locations had higher incidences for disease categories including feet and leg disorders and subclinical mastitis. The phenotypic differentiations in the context of social-ecological heterogeneity might contribute to genetic stratification and G×E along the rural-urban gradient, being the current research topic of the habilitation applicant.

## 3.3. Factors affecting genome-wide associations

Classically, the power of GWAS depends on the amount of LD between SNP and QTL, the QTL effects, the number of phenotypic records, allele frequencies of SNP, and the chosen significance threshold. Furthermore, approaches for adjusting population stratification, types of the dependent variables and number of breeds included in GWAS play an important role. Therefore, the **original research paper 5** compared and evaluated different approaches that can be used to adjust population stratification, with impact on the identification of potential candidate genes, also from a maternal genetic perspective. A further focus was on the evaluation of GWAS in a multi-breed context in the **original research paper 7**.

### 3.3.1. Population stratification

Generally, presence of a systematic difference in allele frequencies between subpopulations in a population contributes to population stratification, with further impact on inflations of false positive associations in GWAS as applied in humans and animals (Kang et al., 2008; Price et al., 2006; Ma et al., 2012). In both human and dairy cattle populations, admixture of individuals with different origins might contribute to population stratification. In modern dairy cattle breeding programs, artificial selection and preferential mating and the worldwide utilization of artificial insemination with same sires, are major causes for population stratification. Therefore,

various approaches, e.g., the genomic control approach focusing on markers not linked with the trait of interest (Devlin and Roeder, 1999), adding principal components (Price et al., 2006), or using a mixed model with polygenic effects (Yu et al., 2006), were suggested to correct the false positive discovery rates. In this thesis, we compared the GWAS results from models with polygenic effects calculated on the basis of selected SNP with principal component analysis (**PCA**). Evaluation criteria were inflation factors and overlaps between significant SNP. In conclusion, the inclusion of polygenic effects with the **G** matrix underestimated the SNP effects. Consideration of the **A** matrix and the leave-one-chromosome-out **G** matrix were not sufficient to correct for the population structure. Based on our evaluations, we suggest the construction of an alternative **G** matrix using all SNP apart from the candidate SNP. However, such strategy implies an extremely long computation time, because the number of alternative **G** matrices, which have to be constructed, is equal to the number of SNP from the genotyped animals. Therefore, from a practical perspective, the direct inclusion of the full **G** matrix might not be the most precise method, but seems to be the most efficient way to correct population stratification.

### 3.3.2. Multi-breed and within-breed GWAS

In order to increase the population size in GWAS, animals from more than one breed were included to conduct multi-breed GWAS (Sanchez et al., 2017; Akanno et al., 2018; van den Berg et al., 2016). Compared to within-breed GWAS, multi-breed GWAS is superior in terms of detecting QTL segregating between breeds, and in mapping precisions. The improvement in mapping precisions can be explained by the shortened extent of LD across breeds (Raven et al., 2014). However, breed-specific QTL might be overshadowed by larger QTL segregating in dominated breeds (van den Berg et al., 2016). Hence, for a deeper evaluation in this thesis, single-breed GWAS were carried out considering the breeds as used in the multi-breed GWAS from the **original research paper 7**. According to the correlations (Table 3), the marker effects from the multi-breed GWAS were in agreement with the effects estimated in black and white dual-purpose cattle (**DSN**), probably due to the domination of DSN cattle in the multi-breed GWAS. Specifically, 39.43% of the genotyped cows were DSN, and the remaining proportions were 23.17% for Simmental, 22.76% for Brown Swiss and 14.63% for Original Braunvieh. The significant SNP from the multi-breed GWAS, *ARS-BFGL-NGS-104430* and *ARS-BFGL-NGS-24800* for rumination, and *Hapmap60738-rs29023086* for not active, were also the top-ranked makers according to their *P-values* in the DSN population. The four significant SNP

for feeding in DSN did not include the significant SNP (*ARS-BFGL-NGS-80066*) from the multi-breed GWAS. Interestingly, although no significant SNP was detected for the behavior "basic active" from the multi-breed GWAS, two and nine breed-specific makers were identified in Brown Swiss and Simmental, respectively. In contrast to "basic active" sharing probably no QTL across breeds, QTL underlying rumination strongly segregated across breeds (van den Berg et al., 2016). Surprisingly, a GWAS for feeding in Original Brauvieh detected 53 significant SNP. However, the SNP were spread over 20 chromosomes, and only one SNP on chromosome 2 surpassed the strong Bonferroni threshold. Furthermore, the quite small population size should be kept in mind when comparing and interpreting results from single-breed GWAS (97 DSN cows, 57 Simmental cows, 56 Brown Swiss cows and 36 Original Braunvieh cows).

**Table 3.** Number of significant markers (according to false discovery rate of 20%) associated with sensor behavior traits and correlations (in parentheses) between marker effects estimated from multi-breed GWAS and within-breed GWAS.

| Trait | Multi-breed GWAS | Within-breed GWAS | | | |
|---|---|---|---|---|---|
| | | Brown Swiss | Black and white dual-purpose | Original Braunvieh | Simmental |
| RUM | 5 | 0 (0.29) | 0 (0.52) | 0 (0.51) | 0 (0.44) |
| FEED | 1 | 0 (0.37) | 4 (0.69) | 53 (0.45) | 0 (0.25) |
| BACT | 0 | 2 (0.38) | not converge | 0 (0.42) | 9 (0.33) |
| HACT | 0 | 0 (0.33) | 0 (0.72) | 0 (0.34) | 1 (0.33) |
| NACT | 1 | 0 (0.42) | 3 (0.69) | 0 (0.30) | 0 (0.37) |
| ET | 0 | 1 (0.36) | 0 (0.65) | 0 (0.39) | 0 (0.37) |
| WEL_IP | 0 | 0 (0.29) | 0 (0.72) | 0 (0.43) | 0 (0.30) |
| WEL_IC | 0 | 0 (0.30) | 0 (0.74) | 0 (0.41) | 0 (0.27) |

RUM = rumination; FEED = feeding; BACT = basic active; HACT = high active; NACT = not active; WEL_IP = welfare index point; WEL_IC = welfare index class.

### 3.3.3. GWAS for maternal genetic effects

Genetically, traits expressed directly at birth and during the short period after birth can be separated into direct genetic and maternal genetic effects. The maternal genetic component represents the ability of the dam to provide a nourishing environment. Pseudo-phenotypes for maternal genetic effects for calving ease and stillbirth in GWAS were de-regressed proofs (Abo-Ismail et al., 2017), daughter yield deviations (Olsen et al., 2010), and predicted

transmitting abilities (Purfield et al., 2015). However, only a limited number of significant SNP and potential causative mutations were identified (Abo-Ismail et al., 2017; Olsen et al., 2010), even for birth weight with a moderate maternal heritability. Therefore, in the framework of this thesis, a simulation study was carried out to investigate the impacts of genetic correlations between direct and maternal genetic effects (**$r_{am}$**), as well as the number and effects of underlying QTL, on the power of GWAS when focusing on maternal genetic effects. The correlation $r_{am}$ strongly influenced the significance of SNP marker effects in GWAS (Figure 2). Surprisingly, no specific conclusion can be made for the effect of the number of QTL. However instead of a gamma distribution with a few major genes, normal distributed QTL effects for maternal genetic effects can barely show any QTL with large effects, which might explain any ambiguities. Moreover, the number of significant SNP was quite constant when $r_{am}$ increased from -1.00 to 0. For $r_{am}$ larger than zero, the number of detected SNP increased apparently. However, negative $r_{am}$ are very common for the calving relevant traits in dairy cattle (Johanson et al., 2011; Eaglen et al., 2012), due to the existence of a direct-maternal environmental covariance (Eaglen and Bijma, 2009) and additional sire or sire × year variation (Robinson, 1996). Even for the ideal scenario of $r_{am}$ = 1.00, averaged over 50 repeats, only 0.5 significant SNP per repeat was close to the true causative QTL. In consequence, detection of QTL for maternal genetic effects remains a challenging task. Further studies addressing the distribution of maternal QTL effects for specific traits and the application of alternative statistical models, are suggested.

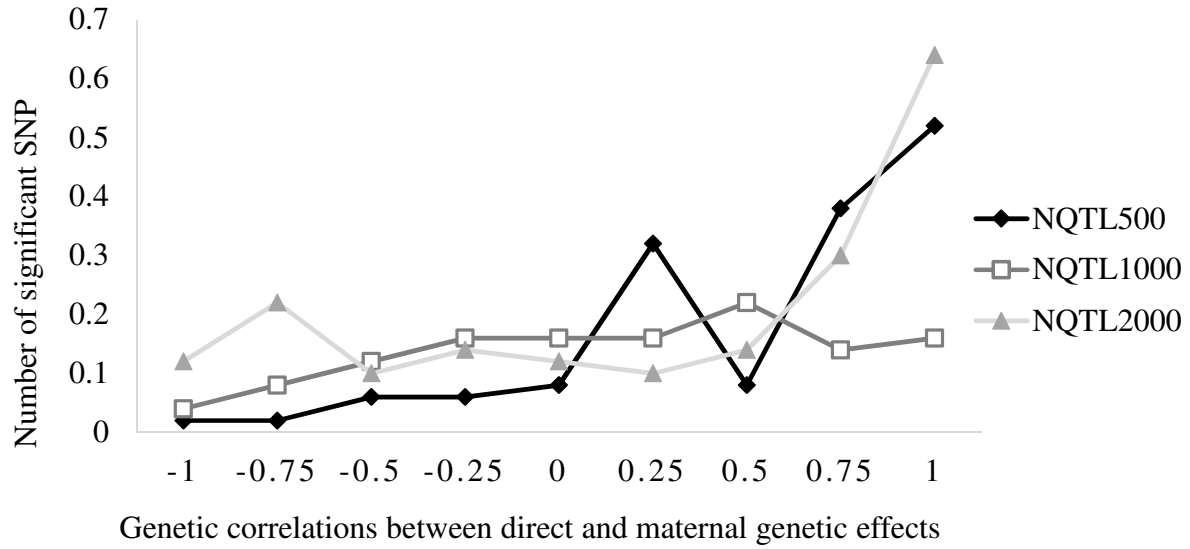


**Figure 2.** Number of significant SNP located within a window of five makers up- and downstream from the true causative QTL for maternal genetic effects in dependency of the number of QTL (NQTL) and genetic correlations between direct and maternal genetic effects.

### 3.4. Genetic and genomic parameters

Variance components and heritabilities estimated on the basis of the **A**, **G** and/or **H** matrices are presented in the **original research paper 2** for production traits, in the **original research paper 5** for body weight, in the **original research paper 6** for growth curve parameters and in the **original research paper 7** for behavior traits. Additionally, the ongoing chapters of this thesis discuss some recent work from Shabalina et al. (2019), focusing on genetic parameter estimates for longevity and health traits from pedigree-based and SNP-based approaches.

#### 3.4.1. Heritabilities

In most cases, SNP-based heritabilities (i.e., **G** matrix construction) were smaller than the pedigree-based (i.e., the **A** matrix construction) heritabilities, irrespective of the genetic architecture. Only for milk yield and fat percentage considering cows in organic farming systems, as well as for rumination and not active in a multi-breed analysis, heritabilities were larger when considering the **G** matrix (but associated with quite large SE). A major explanation for larger heritabilities from the **A** matrix is incomplete LD among SNP from the low- and/or medium-density SNP chips, contributing to insufficient genetic variation captured by the **G** matrix (Román-Ponce et al., 2014). The improved accuracy due to the consideration of the Mendelian sampling term in the **G** matrix was not sufficient to thwart the reduction in accuracy due to incomplete LD between SNP and QTL (Calus et al., 2013).

Modelling the **H** matrix, i.e., combining the **A** and **G** matrices, contributed to the increased heritabilities in the **original research paper 2** for milk yield and SCS, as well as for behavior traits in the **original research paper 7**. In the **original research paper 6**, only the heritabilities for the growth curve parameters representing birth weight were larger when replacing **G** with **H**. For the other growth curve parameters including mature weight, the growth rate and the shape parameter, consideration of **G** in animal models was associated with heritability increases. The SE for heritabilities from the **H** matrix approach ranged from 0.02 to 0.04, but were in the range from 0.05 and 0.10 when replacing **H** by **G**. In the **original research papers 2** and **7**, SE and SD of posterior heritabilities for the same trait were very similar when applying different genetic relationship modelling approaches. Our results from the **original research paper 6** indicate most accurate estimations via **H** applications. In the **H** matrix, usually a weighted genomic relationship matrix ($\mathbf{G}_w$) rather than the simple **G** matrix is included to

depict relationships between genotyped animals (Christensen and Lund, 2010). Since $\mathbf{G_w} = (\alpha\mathbf{G} + (1-\alpha)\mathbf{A_{22}})$, where α is the proportion of variance explained by markers in relation to the total genetic variance, and $\mathbf{A_{22}}$ is the submatrix of the **A** matrix for genotyped animals, the $\mathbf{G_w}$ matrix contains the variances captured by markers and pedigree at the same time (Legarra et al., 2014). Consequently, from our point of view, the heritability increase is in line with the theoretical background when using the **H** matrix.

### 3.4.2. Variance components

The heritability increase when considering the **H** matrix was due to the increase of genetic variance components. For example, in **original research paper 2**, the additive genetic variances for milk yield in the lowest DIM and THI class were 10 $kg^2$ and 5 $kg^2$ when using the **H** and the **A** matrices, respectively. For the low heritability welfare index, the genetic variance increased by 16.67% when modelling the **H** matrix instead of the **A** matrix. Accordingly, Forni et al. (2011) reported smaller additive genetic variances for litter size with the **A** matrix than with the **H** matrix. In their studies, variances for the remaining effects, e.g., permanent environmental effects and residuals, decreased correspondingly, resulting in almost constant total phenotypic variances and in increased heritabilities.

## 3.5. Breeding schemes with focus on genotyped cows and functional traits

For the evaluation of genetic gain, discounted returns and discounted costs in traditional progeny testing and genomic breeding programs comprising production and novel functional traits, Frevert et al. (2014) performed deterministic simulations via ZPLAN+ (Täubert et al., 2010). In the study by Frevert et al. (2014), the breeding goal comprised the six traits milk yield, methane emission, days open, clinical mastitis, body condition score and milking temperament. In the genomic breeding schemes, accuracies of genomic breeding values were varied from 0.20 to 0.80, with increments of 0.20. When assuming equal economic weight per genetic standard deviation for all breeding goal traits, total discounted returns were almost identical for a classical conventional progeny testing scheme and a genomic breeding program assuming accuracies of GBV of 0.2. The substantial reduction in generation intervals was the major parameter explaining the quite large selection responses per year and discounted returns in genomic breeding programs, even when assuming low GBV accuracies. Generation intervals in conventional and genomic breeding programs were 4.97 and 3.17 years, respectively.

Therefore, for an investment duration of 30 years, 6.04 and 9.46 generations were considered in the two programs, respectively. The total discounted return per animal gradually increased from 4.62 Euro to 7.97 Euro, when the accuracy of GBV increased from 0.20 to 0.80. The possible increase of breeding value accuracies in genomic breeding programs is a new opportunity to increase the response to selection for novel traits, such as methane emissions, mastitis, or milking temperament. However, strong selection on most of the functional traits implies antagonistic selection response in milk yield (Gernand and König, 2014; Windig et al., 2006; Pszczola et al., 2019), i.e., a milk yield decrease in the range from 0.45 kg/day to 0.72 kg/day, depending on the GBV accuracies.

In addition to the improvements of genetic or genomic evaluations, it is imperative to derive economic weights for breeding goal traits using scientific methodology, because apart from generation intervals and accuracies, economic weights mainly determine the selection response in individual traits. Generally, it will be a challenge to include properly all new functional traits into overall breeding goals. Further important functional traits which have been ignored in our deterministic calculations (Frevert et al. 2014) are body weight and fat to protein ratio (Coffey et al., 2002; Friggens et al., 2007), milk urea nitrogen (Mitchell et al., 2005; Yin et al., 2012), milk fatty acids (Dijkstra et al., 2011), and the overall health status (Bastin et al., 2011).

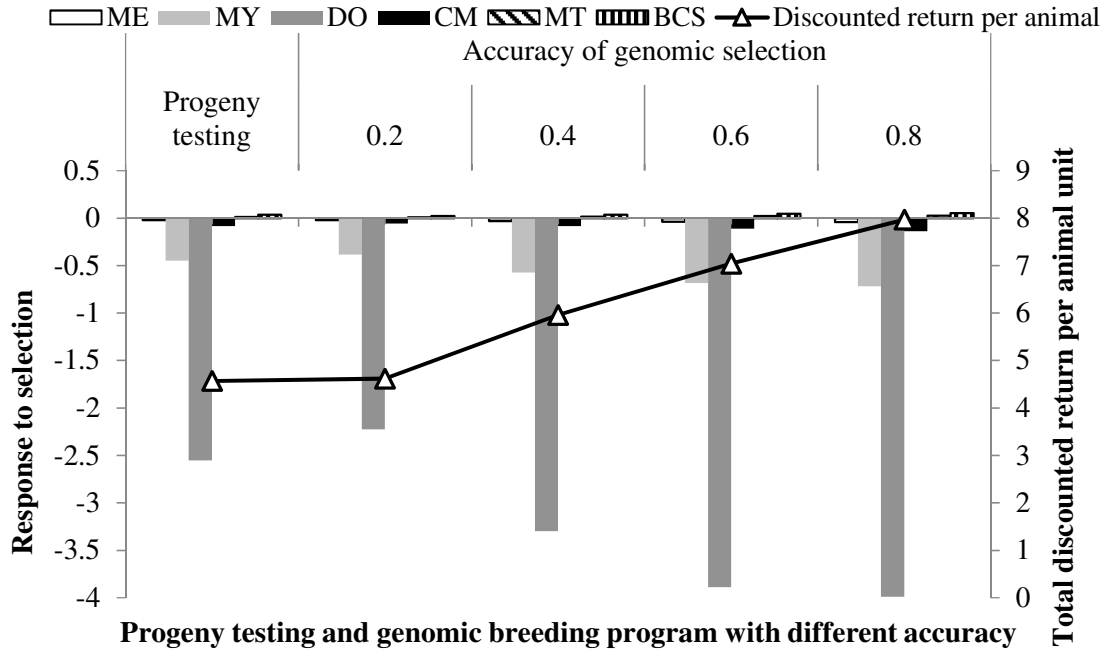


**Figure 3.** Selection responses and total discounted return per animal when assuming equal economic weights per genetic standard deviation for the six breeding goal traits (according to Frevert et al., 2014).

### 3.6. Novel traits for future breeding goals

In recent years, the dairy cattle herd management has been improved stepwise, in order to compensate the genetic deteriorations in functionality (Egger-Danner et al., 2015), to maintain a sustainable production process and to fulfil societal demands (Boichard and Brochard, 2012). Hence, also from a breeding perspective, dairy cattle breeding goals should be extended, with a focus on further novel functional traits. The ongoing attempts in precision farming, which focuses on on-farm data collection and data transfer through the implementation of electronic animal identification, the development of sensor-based data recording, and new communication technologies, contribute to an increasing amount of data (Boichard and Brochard, 2012). In this thesis, precision-farming devices, i.e., electronic ear tags with sensors and automatic weighing systems, were installed in farms to record behavior traits and BW on a longitudinal data basis. Additionally, methane emissions were measured with a portable handheld laser methane detector according to the protocol as defined by Reintke et al. (2020). Further opportunities for CH4 recording are infrared sensors installed in automatic milking systems (van Engelen et al., 2018). In addition to methane, automatic milking systems can also generate dense data for production, behavior and health traits (Santos et al., 2018).

THI as considered in the **original research paper 2** was calculated on the basis of meteorological data from the nearest official weather stations. Aiming on more detailed THI-cow trait associations, on-farm temperature and humidity can be recorded via USB climatic data loggers (Gernand et al., 2019). Furthermore, high-throughput technologies allow to generate large-scale multi-omics data, including genomic data (e.g., SNP and indel), transcriptomic data (e.g., RNA) and proteomic data (e.g., protein sequence and structure), which might help to understand the nature of functional traits in more detail (Vazquez et al., 2016). Meanwhile, consideration of interactions between phenotypic data and multi-omics data being available from the animals (hosts) and microbes in the rumen (Li et al., 2019), specific major pathogens (Mahmoud et al., 2018) and parasitic agents in endoparasite infections (May et al., 2019), might contribute to improvements in the predictive abilities.

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## 4. Summary

This thesis focused on the estimation of genetic parameters for novel functional traits in dairy and dual-propose cattle populations including heat stress responses, methane emissions, longitudinal body weights and respective growth curve parameters as well as electronically recorded behavior pattern. In this regard, quantitative-genetics and genomic modelling approaches were applied. Moreover, genetic architectures for body weight and behavior traits were inferred, i.e., via GWAS for direct-genetic as well as maternal-genetic effects, and ongoing pathway analyses to identify potential candidate genes and their functional annotations. Possible G×E were studied, considering simultaneously continuous time (e.g., aging) and environmental descriptors (e.g., THI). As a novelty, genetic (co)variance components were estimated via RRM on genetic and genomic herd scales, aiming on a deeper understanding of G×E.

In the **original research paper 1**, we stochastically simulated longitudinal phenotypic cow records at five THI levels, as well as genotypes from the 2,000 cows, aiming on the evaluation of the effects of heritabilities, LD, maker density and the proportion of phenotypic records for extreme THI, on prediction accuracies in genomic RRM. As expected, prediction accuracies increased with increasing heritability, LD and SNP density. In order to improve accuracies of genomic predictions, it was imperative to consider a proportion of cows with phenotypic records in heat stress environments (i.e., THI larger than 75), when estimating genomic breeding values of remaining genotyped but not phenotyped cows. In all scenarios, prediction accuracies were larger when modelling the **G** matrix instead of the **A** matrix.

The advantage of RRM considering genotyped cows and heat stress interactions with regard to prediction accuracies for test-day milk yield and SCS was confirmed in **original research paper 2**. Four RRM, i.e., RRM with or without genotyped cattle combined with or without G×E interaction terms, were evaluated using 5-fold cross-validations. The highest prediction accuracies for both traits were identified when applying genomic RRM, and modelling THI as an environmental descriptor. Such modelling superiority was stronger for milk yield than for SCS. For test-day milk yield, a quite large range in genetic correlation estimates for days in milk × THI combinations were identified, indicating GxE interactions. For test-day SCS, genetic correlations were more stable and throughout larger than 0.80. In conclusion, for traits showing sensitive responses to heat stress such as milk yield, it is imperative to include a heat stress indicator in genetic evaluation models, which also contributes to the improved identification of robust dairy cattle in harsh environments.

Genetic parameters for test-day CH4, which were predicted through a deterministic approach as well as an approach combining deterministic equations and stochastic simulations, were estimated on a time scale via RRM in **original research paper 3**. The heritabilities for daily CH4 ranged from 0.15 to 0.37. Genetic correlations between CH4 from different prediction equations were larger than 0.90. Antagonistic genetic correlations in the range from 0.70 to 0.92 were estimated between CH4 and milk yield. Genetic correlations with other functional breeding goal traits were close to zero, but altered in the course of lactation. The simulated data basis for CH4 was used to determine the size of a cow calibration group for genomic selection. A calibration group including 2,581 cows with own measurements for CH4 (a heritability of 0.44 and an effective population size of 100) was competitive with conventional breeding strategies in terms of prediction accuracy.

Genetic parameters and genetic architectures for body weight from different ages were studied in the **original research papers 4** and **5**, respectively. Body weight from birth to calving had moderate to high direct heritabilities. The maternal genetic component was detectable for body weights of calves in the period from birth to an age of 5 months, but later on, maternal genetic variances were close to zero. Body weights for calves and heifers were weakly correlated with production, female fertility and health traits in first parity cow. Genetic correlations between production and fertility traits with insemination body weight were stronger than with birth weight. Considering genomic data of genotyped cows, heritabilities as presented in the **original research paper 5** verified the genetic parameter estimates (**original research paper 4**). Furthermore, in the **original research paper 5**, GWAS for birth weight, weaning weight and body weight at the first insemination date inferred significantly associated SNP and underlying potential candidate genes. With regard to GWAS for maternal-genetic effects, three SNP were significantly associated with birth weight according to the threshold based on 5% false discovery rate. No SNP significantly contributed to maternal-genetic effects on body weight recorded at weaning and at first insemination. Gene annotations identified 76 potential candidate genes for body weight, and these genes were involved in 12 biological processes. Hence, weight development is a very complex biological process, which is controlled by many genes with minor effects.

**Original research paper 6** focused on the estimation of genetic parameters and on prediction accuracies for growth curve parameters from three non-linear growth models, i.e., the Logistic, the Gompertz and the Richards functions, in combination with different kernel similarity matrices. Moderate heritabilities for growth curve parameters confirmed the pronounced

genetic background for body weight with aging. Prediction accuracies for genomic growth curve parameters from different similarity matrices, including two genomic relationship matrices and three kernel matrices, combined with same non-linear functions, were very similar. In combination with all genomic relationship and kernel matrices, model superiority and largest prediction accuracies were observed when fitting the non-linear Richards function.

Heritabilities for novel behavior traits, which were electronically recorded via ear tag sensors, ranged from 0.04 for rumination to 0.20 for feeding and high active. Differences in heritabilities and genetic variances indicate a diverse genetic background for different behavior traits. The underlying genetic mechanisms were unraveled through multi-breed GWAS in **original research paper 7**. According to a very relaxed threshold based on 20% false discovery rate, only five SNP were significantly associated with rumination, one SNP with feeding and one SNP with "not active". The reason for the quite small number of significantly associated SNP with natural cattle behavior might be short-range LD when pooling breeds or limited conserved mutations across breeds. Mendelian randomization based on genomic variants (i.e., the instrumental variables) was used to infer causal inference between an exposure and an outcome. For example, the regression coefficients of rumination and feeding on milk yield were 0.10 kg/% and 0.12 kg/%, respectively, indicating their positive influences on dual-purpose cow productivity. Genomically, an improved welfare behavior of grazing cattle, i.e., a higher score for welfare indices, was significantly associated with increased fat and protein percentages.

The **original research paper 8** depicted possible G×E for production traits and SCS along phenotypic, quantitative-genetic and genomic herd descriptors. Hence, we provided the proof that genetic covariances and correlations between same traits from different herds strongly depend on genetic and genomic herd characteristics, such as inbreeding coefficients or LD within specific chromosomal segments. Apart from the herd variable "allele frequency for the SNP *ARS-BFGL-NGS-4939* within the *DGAT1* gene", genetic correlations between milk yield at minimal and maximal levels for the other descriptors were always lower than 0.6, indicating environmental sensitivity. Especially for low heritability SCS, low genetic correlations were estimated when considering extreme herd classes according to LD of a genomic region on chromosome 6 at herd level, herd size, intra-herd percentage of non-EU sires, and the herd average for non-return rate. Alterations of estimated breeding values of sires in dependency of phenotypic, genetic and genomic herd structures suggest utilization of specific sires for specific herds, indicating further possibilities to optimize mating programs.

**Original research paper 9** addressed the importance of genotyped cows from a commercial herd perspective, including economic aspects and phenotype predictions. For different herd replacement strategies and a herd breeding goal aiming on antagonistically related production and functionality, genetic gain was maximized when focusing on large-scale female cattle genotyping. Especially selection response on the cow-dam pathway increased, through improved replacement and mating designs. From a literature review perspective, the **original research paper 9** discussed further opportunities of cow training sets and commercial cow genotyping. Several studies and theoretical derivations highlighted the importance to including genotyped female cattle in training sets, because cow training sets avoid biased genomic predictions due to intensively pre-selected sires. Additionally, long-term genetic gain in novel functional traits is only possible when implementing the cow training sets. Detection of non-additive genetic effects as well as the control of inbreeding and genetic relationships (to avoid lethal defects) were further arguments to genotype cows in commercial herd.

In conclusion, mainly based on studies using comprehensive datasets for genotyped cows recorded for novel traits, the habilitation thesis presented results for broad genetic mechanisms and identified potential candidate genes for various functional traits. Additionally, G×E were detected along novel continuous “environmental” gradients for both production and functional traits. These findings are important for future improvements of dairy cattle breeding programs, e.g., when expanding breeding goals with further functional trait categories (e.g., behavior traits), and simultaneously considering environmental sensitivity (e.g., heat stress response).