

Ecological, genetic and climatic determinants of range expansion:
a case study on *Ceratocapnos claviculata* in the native
and invaded range.

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In papers 1, 2 and 3, I had the main responsibility for design, field work, data analysis and writing while the co-authors contributed constructive suggestions. Apl. Prof. Dr. R.L. Eckstein and Dr. W. Durka were involved in planning the study. In paper 4, I had the main responsibility for design, field work, data analysis and writing while Dr. W. Durka was responsible for the laboratory analyses. All co-authors contributed constructive suggestions. Apl. Prof. Dr. R.L. Eckstein and Dr. W. Durka were involved in planning the study.

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1. Synthesis

Introduction

Background

Range expansions have been observed in many species during recent decades (e.g. Le Roux and McGeoch 2008). They may be a trace of ongoing postglacial recolonization processes (Taberlet *et al.* 1998). Alternatively or additionally, recent range expansions and invasions in cultural landscapes may be facilitated by processes of global change and thus human activities such as human-mediated long-distance transport, nitrogen inputs, land-use changes and associated disturbances and climate change (Walther *et al.* 2005, Maskell *et al.* 2006, Thuiller *et al.* 2006, Wilson *et al.* 2009). In the following I refer to those aspects relevant for range expansions that were the object of this study.

Invasibility of plant communities and habitat quality

Range expansions imply the colonization and establishment of individuals of species in extant communities. This, in course implies that the ecosystem is vulnerable to invasion (Davis *et al.* 2005). The diversity-invasibility concept (Davis *et al.* 2005) postulates that invasibility is the susceptibility of an environment to colonization and establishment of individuals from species not currently part of the resident community. Establishment, in this context, means that the persistence of colonizing individuals is related to their ability to sustain themselves by gaining access to resources in their new environment. Furthermore, the resulting diversity of the environment is also determined by the diversity of and from the regional species pool. Invasibility of an ecosystem is influenced (hampered or facilitated) by the interaction of biotic and abiotic factors and processes and may vary over time due to changes in the local conditions, events and processes that define invasibility. In a comparative approach proposed by Lortie *et al.* (2004), the interaction of biotic and abiotic factors and processes may be regarded as a series of filters selecting the species of a local community from a larger species pool (Fig. 1). According to the integrated community concept (Lortie *et al.* 2004) the community structure is determined by synergistic (non-linear) interactions among stochastic processes (e.g. dispersal, presence of vectors, distance to new environments), tolerances of species to local abiotic conditions (habitat quality), positive and negative direct and indirect interactions among plants such as competition or allelopathy, and direct interactions with other organisms (e.g. herbivory, pollination).

Resource fluctuation (Davis *et al.* 2000) may be an important factor determining establishment success because new species may be able to colonize during periods of low competition through residents, which may occur after disturbance. Here, degree and frequency of disturbance may determine the invasibility and long term persistence. On the other hand, abiotic environmental filters such as climate and resource availability (Stohlgren *et al.* 1999, Tilman 2004, Davis *et al.* 2005) may display the physiological tolerance of the invader in respect to resource use.

Beside general habitat characteristics, quality and availability of microsites play an important role for the establishment processes (Fenner and Thompson 2005). Contact to substrate, light intensity, temperature, water, nutrients and competition, may meet the species requirements and ensure persistence of populations even if the conditions of the macrosite may seem insufficient (e.g. Price and Morgan 2006). Especially in short-lived therophytic species successful germination and establishment are the key stages in the life cycle that are strongly controlled by those factors (Fenner and Thompson 2005).

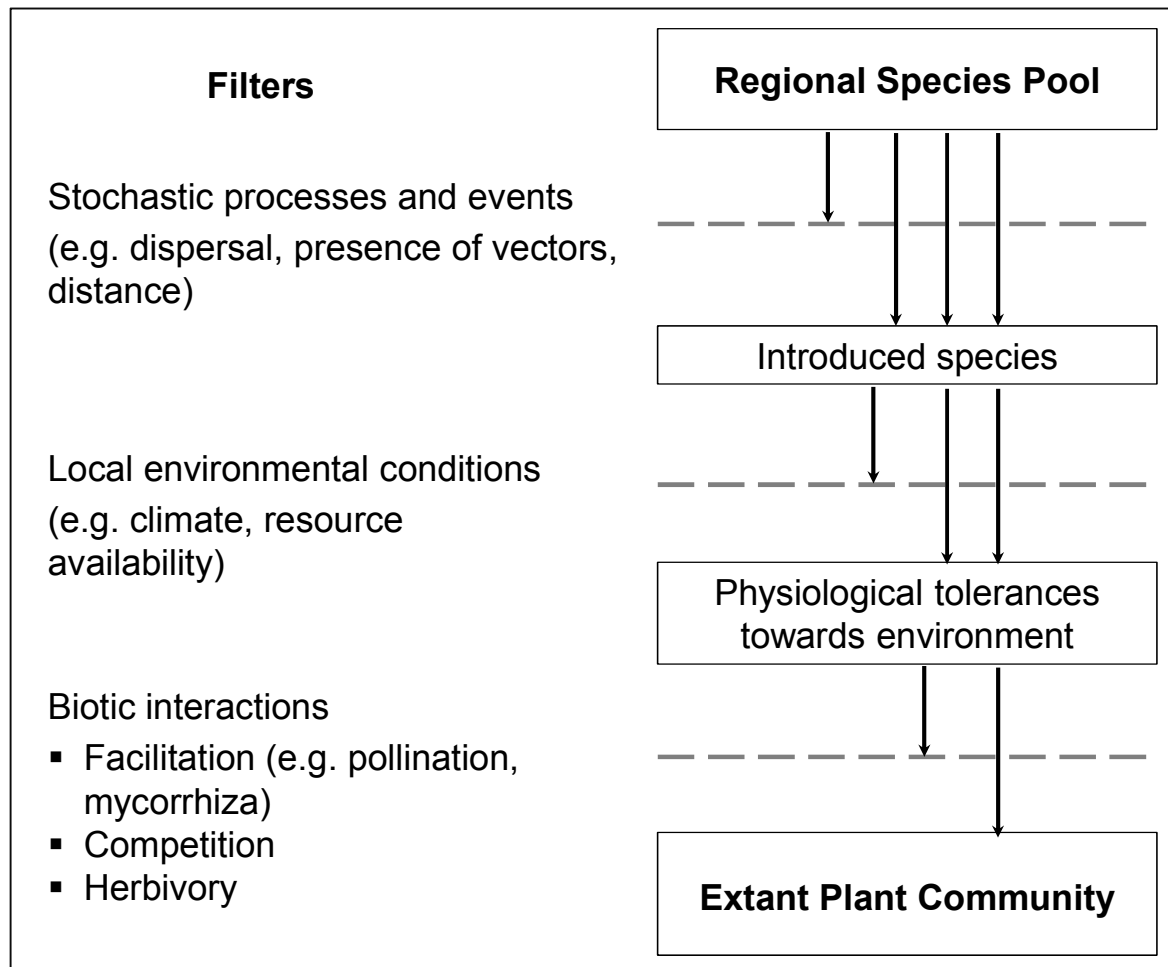


Fig. 1. Simple scheme of the integrated community concept (Lortie *et al.* 2004). The community structure is determined by various filters and processes at a given site, depending on space and time. Arrows depict the movement/invasion of species from the regional species pool through filters (hatched lines).

Local adaption and phenotypic plasticity

The extension of the species range and the colonization of new sites involves that the colonizing species adapt or are preadapted to the local conditions at and beyond the range border. Preadapted species invading new sites have a higher chance to establish successfully (Angert and Schemske 2005, Scott *et al.* 2010). Local adaptation implies that populations of a species often evolve traits that are beneficial under local environmental conditions, but which may not be beneficial under other conditions (Kawecki and Ebert 2004). This may be reflected by, e.g., optimized flowering time and a corresponding higher fitness in the centre of the range compared to beyond the current range (Sagarin *et al.* 2006, Atkins and Travis 2010). Thus, within their range, populations should adapt sufficiently due to the existing selection pressure through abiotic (edaphic, climatic) or biotic (herbivores, pathogens, mutualists) habitat conditions (Joshi *et al.* 2001). Local adaptation may thus lead to homesite advantage, a higher fitness of resident plant species in comparison to introduced congeners. Thus, despite low genetic diversity in the course of founder effects and genetic bottlenecks, species may establish persistent populations. An alternative, not mutually exclusive but complementing strategy to establish successfully in a wide range of habitats represents phenotypic plasticity, which may be defined as given trait's range of phenotypes a single genotype can express as response to environmental stimuli (Nicotra *et al.* 2010). E.g., plant species with a wide spatial or ecological distribution often exhibit high gene flow and/or limited genetic variation (Nicotra *et al.* 2010). In these species, high fitness may be assured by the occurrence of a general purpose genotype, which is able to

maintain a high fitness over a broad range of environmental conditions through compensatory plastic response in morphology and physiology (Schlichting 1986). General purpose genotypes and plasticity can have a fitness advantage in newly invaded founder populations where local adaptation has not occurred yet because of a lack of genetic variation (Bossdorf *et al.* 2005).

Population genetic factors of range expansion

Range expansions may impact intraspecific patterns of genetic variation in the new range, which was exemplarily described for the case of adaptations to local habitat conditions. These patterns may also affect the population dynamics and the ongoing expansion process (Olivieri 2009, Lachmuth *et al.* 2011). Furthermore, patterns of genetic variation in European plant species may reflect the influence of past phylogeographic processes and more recent anthropogenic influences. After the climatic oscillations during Quaternary ice ages, the previous glacial and periglacial areas were recolonized. Across their ranges, many species are characterized by a gradient of high genetic diversity of populations at low latitudes and low diversity at higher latitudes (Hewitt 2000). Populations at the rear, southern edge often could persist during Quaternary oscillations through relatively small altitudinal shifts, and may feature high levels of differentiation among populations and low levels of within population diversity, indicating local adaptation (Hampe and Petit 2005). In contrast, at the northern range margin, repeated range contractions caused extinctions of populations and eliminated genotypes. During postglacial recolonization, rapid expansion by populations from the leading edge and from a limited number of single refugial populations was accompanied by successive losses of alleles along the colonization pathway through repeated bottleneck effects and founder events (Hewitt 1999, Wilson *et al.* 2009, Stewart *et al.* 2010).

Natural and anthropogenic dispersal processes have differently affected patterns of genetic diversity which may influence later establishment success. During natural migrations from the leading edge, species expand by short distance dispersal (Hewitt 2000) or, rarely, by long distance dispersal, which enable colonization of potentially suitable areas distant from the current range (Arrigo *et al.* 2010, Hampe 2011). However, due to the rarity and stochasticity of successful long distance dispersal, populations in the new range are expected to show strong founder effects and may diverge genetically from source populations (Ibrahim *et al.* 1996). In contrast to natural dispersal pathways, human mediated dispersal processes tend to introduce larger numbers of propagules from more diverse or different sources (multiple introductions) over shorter periods of time (Roman and Darling 2007, Simberloff 2009, Wilson *et al.* 2009). In this case, the genetic diversity in introduced populations of invasive species often has been found to be equal to that of native populations so that no pronounced genetic bottlenecks were detectable or multiple introductions may have compensated for losses of genetic diversity during expansion (Bossdorf *et al.* 2005, Roman and Darling 2007, Lachmuth *et al.* 2010). Thus, propagule pressure, the number of introduction events and the source regions, as well as the genetic structure within the source region are decisive factors for genetic diversity and establishment in the target region and should be taken into consideration when studying range expansions.

Plant breeding systems have a strong influence on both, genetic diversity of populations and the population dynamics and thus may have an impact on expansion success (Hao *et al.* 2011). Self-fertilizing species with short life cycles such as many weeds are often successful and fast colonizers. If only a low number of diaspores is introduced, the ability for self-fertilization is advantageous for fast population growth because neither compatible partners nor pollinators are needed (Barrett *et al.* 2008). In contrast, reduced genetic diversity in selfing species may also have long-term negative impacts due to a reduced evolutionary potential for adaptation to changing environments (Leimu *et al.* 2006, Olivieri 2009). However, a number of successful large scale invasions despite of very low genetic

variation have been reported (e.g. Novak *et al.* 1991, Hollingsworth *et al.* 1998, *Pennisetum setaceum*: Le Roux *et al.* 2007, *Ferula loscosii*: Perez Collazos *et al.* 2009, Grimsby and Kesseli 2010, *Eichhornia crassipes*: Zhang *et al.* 2010). The success of those populations might be based on either niche matching (Pérez *et al.* 2006) or on the presence of general purpose genotypes, which exhibit high phenotypic plasticity (Richards *et al.* 2006, Roman and Darling 2007).

Model Species

The annual forest herb *Ceratocarpus claviculata* (L.) Lidén (chapter 3 Fig. 5) belongs to the family Fumariaceae DC. and is, like many weeds, a tetraploid ($2n = 4x = 32$) (Lidén 1986, Klotz *et al.* 2002). The genus *Ceratocarpus* is characterized by the architecture of the style (Ryberg 1960, chapter 3 Fig. 3). It is an herbaceous, slender and, due to its tendrils, climbing plant species. *C. claviculata* is characterized by fibrous roots, sympodial growth, yellowish flowers, many seeded fruits and a deciduous style (Fedde 1960).

Due to the weakly developed supporting tissue, the more or less branched stem is very weak, but can still reach up to almost 200 cm in length. The ability to climb enables the slender plant species to reach heights with better light conditions which may be advantageous in sites with well developed herb/grass layers (Ellenberg and Leuschner 2010).

Usually the species is in bloom between June and September, which is about two to three months after germination (e.g. Jäger and Werner 2005, chapter 3). The flowers are organized in simple, bracteous, open racemes of 5-8, ca 6 mm long, zygomorphic flowers. Like most other species in the tribe *Fumarieae*, *C. claviculata* is a synoecious, homogamic, self-compatible and autogamous species. But it produces nectar and is therefore visited and pollinated by honey- and bumblebees (Lidén 1986, Klotz *et al.* 2002, chapter 3). *C. claviculata* produces in average 2 seeds per flower of ca. 1.8 mm length (Brückner 1984). Seeds feature a very small ariloid (Ryberg 1955). The species propagates by seeds only and dies off after a large part of the seeds are mature and have been dispersed with or from the capsule.

C. claviculata is predominantly a summer- but also winter-annual semirosette therophyte (chapter 3). In climate chamber experiments, germination was highest after stratification at 4°C ($35.0 \pm 2.7\%$) compared to stratification at 0°C (23.7 ± 1.9) or 15°C (12.4 ± 1.1 ; ANOVA: $F_{2,90}=43.58$; $p < 0.001$; Chrzan *et al.* unpublished data). These results confirm that the peak of germination occurs in spring after break of seed dormancy by low temperatures during winter. A small fraction of seeds does not need cold pre-treatment and thus, may already germinate in autumn (September), shortly after dissemination.

In the northern part of its native range, most populations occur in semi-shaded open oak-, birch-, and pine-forests, in fringes, hedgerows or ditches along roads. However, the species also thrives very successfully on sun exposed forest clearings (Buchenau 1861, Tüxen and Jahns 1962, Buttler 1986, Fukarek and Henker 2006). *C. claviculata* usually occurs on fresh soils of intermediate moisture (Lethmate *et al.* 2002) on humic, acidic and moderately acidic soils (pH ca. 3.5-4.6) of intermediate to high fertility (Ellenberg *et al.* 1992, Hill 2004). The species roots in the organic surface layer, which consists mostly of moder or raw humus, or of hardly decomposed litter formed by needles, and leaves of trees or grasses (Passarge and Hofmann 1968, Lethmate *et al.* 2002). In the Temperate floristic zone, *C. claviculata* occurs from the planar to the colline altitudinal zone, yet is predominantly confined to lowlands. In the submeridional floristic zone, a clear shift toward montane habitats is visible (chapter 3 Figs. 1 and 4).

The species has been regarded a eu-oceanic species due to its distribution pattern in Western Europe (Jäger and Werner 2005). However, during the last decades the species showed both an increase in

frequency within its range such as in the UK (Hill *et al.* 2004), in northern Belgium (Vannerom 1994) and north-western Germany (Jäger and Werner 2005), and a rapid range expansion east- and northwards into sub-oceanic and northern-temperate regions. It established in northern France (Decocq 2000), north-eastern North Rhine Westphalia (Pollmann and Lethmate 2006), Mecklenburg-Western Pomerania (Meyer and Voigtländer 1996), north-eastern Lower Saxony (Dengler 2007), north-western Saxony-Anhalt (Passarge and Hofmann 1968, Rattey 1984) and Brandenburg (Benkert *et al.* 1995) in Germany, as well as in Skåne and Blekinge in southern Sweden (Hylander 1971, Oredsson 2005, chapter 3 Fig. 1). A common character of all these sites is that they originally have been acidic beech or birch-oak forests but now changed to rather non-natural (e.g., stands of *Pseudotsuga*, *Pinus*, *Larix*, *Robinia*) or non-constant forest communities, clearings or disturbed sites (Pott 1995, Benkert 1996, Pollmann and Lethmate 2006, Ellenberg and Leuschner 2010). Additionally, the species occurred in most of the sites spontaneously, often with distances of 10s to 100s km from the next population and in higher abundance than in native range sites (chapter 3 Fig. 1).

Several competing but not mutually exclusive hypotheses have been put forward to explain the recent spread of *C. claviculata* (Lethmate *et al.* 2002). These are closely related to the sequence of factors and filters such as dispersal, genetic diversity, environmental filters, and habitat quality that determine the invasibility of a local community (Lortie *et al.* 2004, Davis *et al.* 2005): (i) The Dispersal/“*hemerochory-hypothesis*” is based on the observation that the seeds of *C. claviculata* lack structures facilitating long-distance dispersal, and that new populations often have been discovered in great distance to the nearest potential source population. Consequently, anthropogenic activities such as transport of wood and forest management may be responsible for the fast regional expansion and local spread (Buttler 1986, Benkert *et al.* 1995, Decocq 2000, Lethmate *et al.* 2002, Horstmann 2005, Oredsson 2005). Many herbaceous species may be transported with soil around the roots of forestry saplings (Prach *et al.* 1995). (ii) The increased winter temperatures/“*mild-winters-hypothesis*”: after seeds have been dispersed to a new locality, mild winter temperatures (as a consequence of, e.g., climate change) may facilitate seedling survival and the establishment of populations (Folland and Karl 2001, Lethmate *et al.* 2002). (iii) The nitrogen availability/“*nitrogen-deposition-hypothesis*” claims that increased atmospheric nitrogen inputs may increase the performance of this species after successful establishment (Pott and Hüppe 1991). In fact, during the last 50 years nitrogen availability in soils increased as a consequence of atmospheric deposition (Bobbink *et al.* 2010). Growth and competitive effect of *C. claviculata* seems to be promoted by this process (Lethmate *et al.* 2002), as it was one of nine forest understory species that showed a significant increase in coverage between 1984 and 1993 in parallel with increased nitrogen deposition (Van der Eerden *et al.* 1998).

Study questions and aims

The main goal of this study was to assess the filters, traits and processes which are related to the expansion/invasion success and persistence of colonizing plant populations in native and neophytic range sites of the model species *C. claviculata* by means of common garden, greenhouse and climate chamber experiments. To this end, the project focused on the following objectives:

- a. To study the biology, community composition, habitat requirements and the effects of ecological filters for the colonization of *C. claviculata* across the entire range. (chapters 2, 3 and 4).
- b. To test for the phenotypic plasticity and local adaptation in native and neophytic populations (chapter 4 and 5).
- c. To analyze population genetic patterns (diversity, founder effects) in the native and neophytic range and characterize possible dispersal pathways (chapter 5).

Study area

For the population genetic study and one part of the community composition study we sampled across the entire native and neophytic range of *C. claviculata* (Fig. 2). The second part of the community composition study and all germination experiments were carried out in the main study area, which comprises eastern Netherlands (NL), north-western Germany (NWG) as parts of the original range, and north-eastern Germany (NEG) and southern Sweden (S or S1) in the neophytic range (Fig. 2)

The native and neophytic main study area differed strongly with respect to continentality (chapter 2 Table 1). The neophytic range is part of the Continental zone (Metzger *et al.* 2005); the study sites in the native range belong to the Atlantic North zone. However, during the past years, temperature had increased and precipitation decreased which resulted in warmer winters but drier vegetation periods in all four main study regions (DWD 2010, SMHI 2010).

Soil and substrate types differed only slightly between the four study regions with sand dominating in NL, NWG and NEG and sand to silt in S. In all regions, the predominating humus types were moder and mor. Mean pH_{KCl} in the upper 10 cm of the soil in selected sites was in average between 3.2 and 3.3 (chapter 2).

Chapter outline

This thesis is based on four manuscripts. Three of them have been published in peer-reviewed international scientific journals. The third manuscript has not been submitted yet. This section gives an outline of the contents and methods of the manuscripts (chapters 2-5). Main results and conclusion are presented in the next section.

Chapter 2: *Vegetation databases as a tool to analyze factors affecting the range expansion of the forest understory herb Ceratocarpus claviculata*

To gain deeper understanding of factors affecting the invasibility of plant communities with *C. claviculata*, we studied the community composition and cover of *C. claviculata* as a fitness parameter on three different scales using ordination techniques (CCA, DCA): i) we compared community composition across the entire range, ii) we analyzed whether abundance of *C. claviculata*, community composition and habitat quality varied between the native and the invaded range, and since the species has also increased within the native range, we iii) analyzed changes of abundance of *C. claviculata*, community composition and habitat quality in mixed oak forests during the past decades within the native range. For this study, we sampled own vegetation relevés in the main study regions north-western Germany, north-eastern Germany and southern Sweden (Fig. 2), employed various vegetation databases to obtain relevés with *C. claviculata* from across the entire native range and received Dutch relevés from before 1970 and between 1990 and 2006 for the temporal approach.

Chapter 3: *Biological Flora of Central Europe: Ceratocarpus claviculata (L.) Lidén*

In this paper, I reviewed the taxonomy, morphology, distribution, habitat requirements, life cycle and biology of *C. claviculata*. For this, I compiled all available data of the species to these topics and generated own data when necessary. I carried out vegetation surveys in the main study area and designed small greenhouse or climate chamber experiments with the species (such as influence of litter cover and type on germination success, relative importance of cold stratification and germination temperature for seedling emergence). In order to avoid maternal effects, in all experiments I used seeds from greenhouse cultivated plants.

Chapter 4: *Homesite advantage and phenotypic plasticity in a range expanding forest therophyte*

To test for the presence and effects of local adaptation and climatic factors on establishment and fitness, I carried out a reciprocal seeding experiment in the native range (north-western

Germany=NWG and The Netherlands=NL) and in the neophytic range (southern Sweden=S and north-eastern Germany=NEG), respectively (Fig. 2). Additionally, we carried out a seeding experiment along a gradient of increasing continentality at two sites beyond the current range (S2 and S3) in order to detect climatic limitations of potential expansion processes (Fig 2). In a common garden experiment, I analyzed the effects of founder (seed) origin and habitat quality (filters: sun exposure and nitrogen availability) on the establishment of *C. claviculata*.

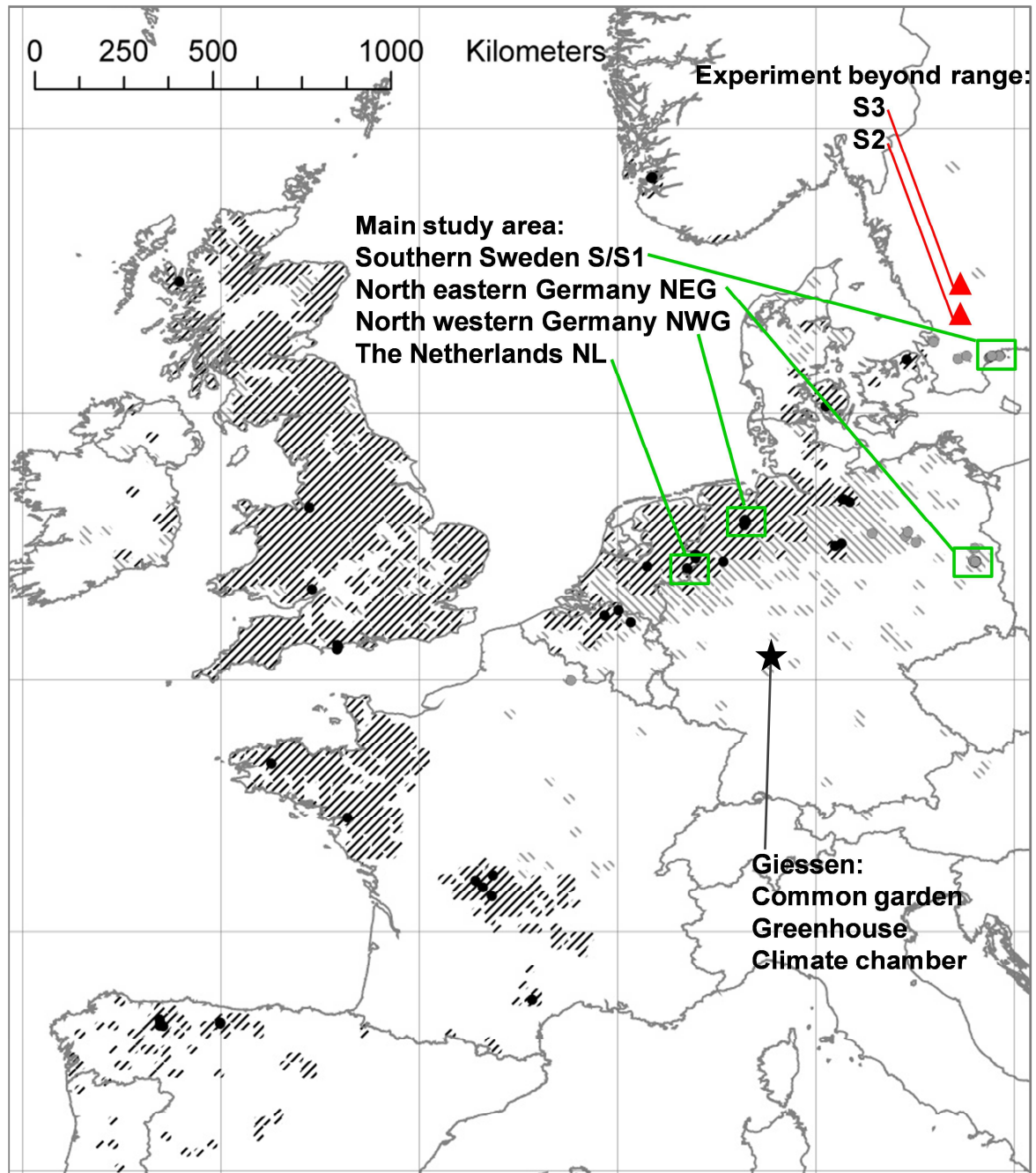


Fig. 2. Entire range (black-hatched=native, grey-hatched=neophytic range) of *C. claviculata*. Dots mark all sampled locations in the native (black dots) and neophytic (grey dots) range. Squares, triangles and star depict sites of experimental set ups.

Chapter 5: Range expansion of a selfing polyploid plant despite widespread genetic uniformity

In this paper I investigated patterns of genetic diversity across the native range to assess the current population structure and phylogeographic patterns. I then tested whether genetic diversity is reduced in

the neophytic range and tried to identify source regions of the expansion. Plant tissue was collected from 55 populations in the native and 34 populations in the neophytic range (this chapter Fig. 2 and chapter 5 Fig. 1). Amplified Fragment Length Polymorphism markers (AFLP) were used to analyze the genetic variation and the population structure. Population differentiation among populations was assessed by means of Wrights F -statistics and analyses of molecular variance (AMOVA). Finally, I studied pollen-ovule ratio as a proxy for the breeding system.

Main results and conclusions

Habitat requirements and filters for colonization

Analyzing the cover of *C. claviculata* across the entire range I found that there was a huge heterogeneity in the dataset (chapter 2). To a large amount this was associated with actual environmental heterogeneities, but might also reflect differences in sampling methods and intention, as I used own vegetation plot data as well as relevés gathered by other scientists in vegetation plot databases. However, a remarkable portion of variation in the dataset was due to the effect of the climatic environmental differences after accounting for the effects of tree cover and plot size. The climatic environmental zones largely depend on minimum and maximum temperature of the months January, April, July, and October, on precipitation during these months and on oceanity (Metzger *et al.* 2005). As expected from the wide dispersal of the species in a huge number of habitats, climatic stratification accounts for a large proportion of species composition. Especially relevés from the central part of the range indicated a large floristic heterogeneity; very likely because the central range comprises a variety of favorable environmental habitat conditions.

At the smaller scale, particularly across the sites in the main study region (NWG, NEG, S) a comparison of the vegetation in the native and neophytic range revealed that differences in community composition between native and neophytic range sites were as large as between the neophytic range sites. This suggests that *C. claviculata* has successfully established in various vegetation types that differ among the studied regions. However, there were no systematic differences in habitat characteristics between native and neophytic range sites. Mean cover of *C. claviculata* in the neophytic range was higher. This is surprising since the neophytic regions are, despite milder winters during the past decade, still characterized by continental climate. Furthermore, in all regions many individuals were observed that germinated apparently in autumn and survived despite low winter temperatures probably in protected microsites, even after the long and cold winters in 2008 and 2009. On the other hand, in the reciprocal seeding experiment *C. claviculata* (chapter 4) showed a fitness advantage with respect to germination and establishment in the native range compared to the neophytic range. This is in line with many other studies (e.g. Angert and Schemske 2005, Giménez-Benavides *et al.* 2007).

Fitness advantage in the native range of *C. claviculata* may be related to differences in habitat quality in the study regions: continentality, tree cover and litter layer increased gradually from The Netherlands, north-western Germany, Sweden to north-eastern Germany, whereas north-western Germany and Sweden were floristically most similar compared to all other regions (chapter 2). However, besides climatic differences we found that native sites featured a higher litter cover which, in turn, seems to have a positive impact on germination, establishment and length of *C. claviculata*. Presumably, seedling emergence and growth is facilitated under litter by attenuating climatic extremes such as cold or drought (e.g. Facelli and Pickett 1991, Eckstein and Donath 2005, Loydi *et al.* 2013). In contrast, germination was inhibited in sites with high covers of bryophytes or herbs (chapter 4). These results were also observed in greenhouse experiments (Peppler-Lisbach *et al.* unpublished). Germination success of *C. claviculata* was highest if there were suitable microsites such as moderate moisture keeping leaf litter layer and if seeds had contact to the soil or humus layer (chapter 4).

Our observations correspond to the results of a reciprocal study on the three annual species *Biscutella didyma*, *Bromus fasciculatus* and *Hymenocarpus circinnatus*, where local environmental conditions had larger effects than regional climate (Petrů and Tielbörger 2008). These findings may explain that despite large climatic differences between native and invaded ranges, there are partly only small floristic differences and similar results for fitness/establishment of *C. claviculata* in Sweden and north-western Germany (chapter 2).

However, northward range expansion as a response to recent climate change has been reported in a number of plant species (Thuiller *et al.* 2006). The northern range margin of the atlantic species *Ilex aquifolium* goes parallel with the 0°C isoline. During the past decades this isoline shifted northwards which could also be observed for the northern margin of *I. aquifolium* (Walther *et al.* 2005). For *C. claviculata*, which has a similar distribution as *I. aquifolium*, the recent range expansion also has been related to milder winters during the last decades (Lethmate *et al.* 2002). In fact, the geographic area of the climatic niche of *C. claviculata* has expanded and climatic conditions in the native and neophytic range are very similar with only slight increases of temperature differences between winter and summer as well as the proportion of summer rain towards the recently colonised range (chapter 3). Similar to other species, this might indicate that the current distribution is probably not in equilibrium with the climatic niche yet, which might suggest further range expansion (Magri *et al.* 2006, D'Andrea *et al.* 2009, but see chapter 4).

C. claviculata belongs to the forest species, which benefit during invasion and colonization process from browsing animals, game passes and resting places through the creation of a less dense vegetation layer and soil disturbance (e.g. Förster 1998). All these conditions may facilitate germination and establishment of the therophytic *C. claviculata*. Also, forestry and human leisure activities, roads and paths may have positively affected both the dispersal of *C. claviculata* and the disturbance of forest sites (Buttler 1986, Pott 1995, Dzwonko and Loster 1997, Tendler and Peppler-Lisbach unpublished results). Locations with intensive forestry management and clearfellings such as in conifer-dominated forests also exhibit disturbance of the vegetation layer and soil surface, but usually over much larger areas. Germination and establishment of ruderals and light-demanding species such as *C. claviculata* are also promoted in these communities (Berg 2004, Dengler *et al.* 2007). In fact, own results confirmed that abundance of *C. claviculata* was associated with disturbance through silviculture, which, in fact, was greater in the new range (chapter 2). A larger human impact in the new range might also be reflected by a higher proportion of hemerobic and ruderal species indicating a higher availability of microsites for the establishment of short lived species, and by higher proportions of neophytic and nitrophilous species (Lososová *et al.* 2006, Chytrý *et al.* 2008, chapter 2). Many species entering disturbed habitats have weed-like traits, such as the annual, self-pollinating *C. claviculata*. It was suggested that the often observed higher abundance of introduced species in their new range is due to a higher rate of disturbance there, which creates colonization gaps with higher substrate availability and low competition (Davis *et al.* 2000, Chmura and Sierka 2007, Fausch 2008).

As *C. claviculata* has no adaptation to long distance dispersal, fast expansion over long distances of tens or hundreds of kilometers and occurrence of isolated populations in neophytic sites may be overcome by direct anthropogenic dispersal vectors such as transport of wood and forest saplings from tree nurseries and afforestation (Buttler 1986, Decocq 2000, Oredsson 2005). Large amounts of pulpwood and sawtimber were transported to Swedish sawmills and paper plants after severe storms in north-western Germany and The Netherlands in the 70s. Theses transports most probably facilitated the introduction of *C. claviculata* to Sweden (Buckley *et al.* 2003, Oredsson 2005). Thus, anthropogenic impact may directly facilitate seed dispersal and provide sites and resource conditions suitable for germination and establishment of *C. claviculata*. Moreover, expansion in the new range might be related to an expansion within the native range. An increased propagule pressure in the native

range caused by, e.g., more favorable nutrient supply or climate, may result in an increased dispersal probability and thus provides a further explanation for successful expansion (chapter 2).

The temporal comparison of Dutch relevés revealed that the cover of *C. claviculata* decreased significantly between the two time periods (chapter 2) despite an increase of indicators for nutrients and an increased anthropogenic impact on the studied forest community. This differs from my other results as well as results of pine forests (van Dobben *et al.* 1994). However, in this investigation I focused on deciduous forests, which are less intensively managed, especially after the abandonment of traditional deciduous woodland management techniques (Rodwell 1998).

Phenotypic plasticity and homesite advantage

The paradox of successful colonization (chapters 2 and 3) and limited genetic variation (chapter 5) suggests the occurrence of general-purpose genotypes and phenotypic plasticity. *C. claviculata* shows moderate to pronounced phenotypic plasticity (chapter 4) as we detected plastic responses with respect to the changing light conditions (e.g. leaf morphology) and nitrogen (e.g. biomass, in field observations and common garden factorial experiment). Phenotypic plasticity probably facilitates the colonisation of various habitats by *C. claviculata* (see chapters 3 and 4). Examples of phenotypic plasticity have been observed in many widespread species (e.g. Loomis and Fishman 2009, Nicotra *et al.* 2010). These properties may have a fitness advantage in founder populations where local adaptation has not occurred yet because of a lack of genetic variation (Bossdorf *et al.* 2005), or as a strategy to respond adequately to different filters of habitat conditions despite low genetic variation (chapter 4).

Phenotypic plasticity may be enhanced by processes conveying intragenomic variation, like polyploidy or epigenetic variation, which may be particularly relevant for the success of species during range expansion (Prentis *et al.* 2008, Pandit *et al.* 2011). In both invaded regions *C. claviculata* is present for about 30 years in numerous, sometimes huge populations (chapter 2). Thus, despite low genetic variation mostly in the northern half of its range, the tetraploid *C. claviculata* may apparently buffer environmental variation at least partly through general purpose genotypes and phenotypic plasticity, probably induced by intragenomic diversity and fixed heterozygosity (chapters 4 and 5).

In contrast, individuals of different seed origins showed different phenotypes under equal habitat conditions (chapter 4). This might indicate genetic differentiation and might have evolved through adaptive processes to specific environmental conditions or genetic erosion in the new range during the colonization process (chapter 5). Additionally, we detected significant interactions between several “origin” × “habitat quality” combinations (factorial common garden experiment) and homesite advantage for establishment of *C. claviculata* for both, native and, to a smaller degree, neophytic sites (reciprocal seeding field experiment, chapter 4). Homesite advantage in fitness-relevant traits and interactions between the factors origin and habitat quality may indicate local adaptations to the habitat conditions of a site (Potvin and Tousignant 1996, Joshi *et al.* 2001, Nicotra *et al.* 2010). Since introduction of *C. claviculata* in the neophytic range, only 30 to 40 years have passed, which is probably a too short time span for the evolution of adaptations. Adaptation to local environments and beyond the range margin may actually be hindered due to high gene flow, genetic drift or generally low genetic variation (Kawecki and Ebert 2004). Comparable to *C. claviculata*, other species (*Mimulus cardinalis* and *M. lewisii*) featured highest fitness in the range centre, reduced fitness at the range margin and zero fitness when transplanted beyond their present range limits (Angert and Schemske 2005). There were weak differences in performance between the populations of each species. Thus, border populations have probably not yet acquired the right mutations to exhibit high fitness and to extend the border (Angert and Schemske 2005). In fact, north-eastern German

populations lacked genetic diversity more than any other region and there was practically no differentiation between populations (chapter 5).

Concerning further expansion, the neophytic range is still within the physiological and ecological limit of the species. However, under more continental climate, *C. claviculata* was not able to establish beyond its current range (chapter 4). The seeding experiment in the two regions in Sweden beyond the current Swedish range showed that further dispersal may be limited by climatic and ecological factors. Though there was some seedling emergence, the species failed to establish in these sites which might be due to late frost during spring as well as drought, which may hamper germination/establishment. Additionally, in those regions the proportion of bryophytes and lichens increase and the proportion of leaf litter decrease. The former were inhibiting germination during experiments, the latter facilitating germination. Thus, for the studied Swedish region further expansion into more continental sites seems unlikely and the species seems to be in equilibrium with its potential distribution. There, where the species has not reached its physiological range margin, further expansion might be possible (see above “Habitat requirements”). Apart from higher continentality, abiotic and biotic conditions such as lower nutrient supply, higher proportions of dwarf shrubs, bryophytes and lichens might be filters which hamper establishment and further expansion of the species.

Population genetic factors of range expansion

Generally, genetic diversity at population level was very low (mean $H_e=0.004$) and only two (multilocus) genotypes dominated large parts of the range and most of all the new range (chapter 5). These results and a low pollen-ovule ratio are consistent with an autogamous breeding system. Genetic variation decreased from the native to the neophytic range. Within the native range, it decreased towards north and east (chapter 5 Fig. 2), which represents a decrease of genetic variation with distance to the Pleistocene refugia, Spain. In contrast, population size increased and tended to be higher in the neophytic sites. Bayesian analysis of population structure revealed six clusters with a clear geographic pattern (chapter 5 Fig. 1). Two clusters, which comprised mostly samples from the south and east of the range, harboured by far the largest number of genotypes and showed higher genetic variation, while the more northern clusters had the lowest variation. Putative source regions of the neophytic range are situated in north-western Germany and adjacent regions. Population differentiation was strong ($F_{ST}=0.812$) as expected from low levels of within population variation. Hierarchical AMOVA showed that differentiation between the native and neophytic range accounted for 13% of variation, whereas most variation resided among populations within ranges (65%, chapter 5 Table 2). We found higher differentiation in the native range than in the neophytic range due to low levels or lack of variation in the latter.

This is in line with a general pattern in many species of a latitudinal cline of genetic diversity from southern Europe to northern Europe (Hewitt 1999, Hampe and Petit 2005). Lower genetic diversity in the northern parts of the range may be related to postglacial recolonization processes. Due to climatic oscillations during the Quaternary, range contractions and expansion have eliminated genotypes in northern Europe. During recolonization of the northern European sites, a loss of alleles may be the result of rapid expansion of already impoverished populations (Hewitt, 1999), and founder effects and an autogamous breeding system may have enhanced genetic erosion (e.g. Durka 1999, Prentis *et al.* 2008). Thus, patterns of genetic variation in the native range of *C. claviculata* do not follow the central-marginal model, but show a rear edge vs. leading edge pattern.

The current distribution of *C. claviculata* and models of the distribution of climatic zones during the Pleistocene suggest that the south-western range (Iberian Peninsula and France) served as refugium (chapter 3) which is corroborated by the comparatively high levels of genetic variation found in this region (chapter 5). This has also been proposed for other species such as the oceanic orchid

Himantoglossum hircinium (Pfeifer *et al.* 2009) and the European-wide distributed *Corylus avellana* (Palmé and Vendramin 2002).

Despite losses of genetic variation during postglacial recolonization and low dispersal ability, the species expanded successfully during the past 40 years over large distances to southern Sweden and north-eastern Germany, where it occurs as an opportunistic neophyte (chapter 5). Recent massive anthropogenic impact facilitated invasibility of plant communities and accelerated expansion of the forest species *C. claviculata* (chapter 3). Results from Bayesian cluster analysis supported the assumption that seeds of *C. claviculata* might have been introduced through wood transport after heavy storms in the 70es from north-western Germany and The Netherlands to Sweden as these regions showed the same clustertypes (chapter 5).

In general, genetic impoverishment especially in the neophytic range may be due to founder events and genetic bottlenecks during the invasion process (Barrett and Kohn 1991, Edwards *et al.* 2006, Okada *et al.* 2009). However, it is more likely that in the case of *C. claviculata*, pre-existing low levels of genetic diversity in the source populations are responsible (chapter 5). Furthermore low genetic variability may limit colonization of new habitats due to lack of evolutionary potential under variable or novel environmental conditions (Stebbins 1957). However, according to the numerous populations in the neophytic range and an increase of population size towards the neophytic sites, *C. claviculata* is a successful colonizer despite of very low levels or lack of genetic diversity.

Conclusions

Across its entire range *C. claviculata* occurs in a wide range of habitats. However, during the past 40 years the species expanded its range successfully, despite lacking mechanism for long distance dispersal, towards north and east to more continental southern Sweden and north-eastern Germany. There, populations have even established with higher abundance than in the native range sites, despite extremely low genetic diversity. Generally, abundance increases towards the north-eastern range border, whereas genetic diversity decreases.

This paradox could partly be explained against the background of global change. Firstly, due to missing own dispersal mechanism, successful dispersal must have been associated with anthropogenic activities such as intensification of forestry and wood transport. Moreover, these types of activities often contribute accidentally to improved germination and establishment conditions for the ruderal species by disturbing the present herb layer and soil surface. Secondly, climate warming during the past decades facilitated invasion to subcontinental neophytic range sites. In this context, ecologically suitable microsites for germination which hamper climatic extremes and attenuate macroclimatic effects may also have contributed to the establishment success.

Once introduced, *C. claviculata* may easily reproduce due to its short life cycle and the ability to self-pollinate. Furthermore, the species may be supported through locally increased nutrient supply by a higher propagule pressure. Certainly, low genetic diversity and self-pollination may lead to inbreeding depression. However, as the species probably features general purpose genotypes and high phenotypic plasticity with respect to germination (winter and summer annual), light, and nutrient conditions, these may facilitate establishment in new range sites.

In contrast, there are some limiting factors for further expansion: Firstly, in sites with dense vegetation cover it is difficult for *C. claviculata* to establish. Secondly, the seeding experiment beyond the current range showed that further dispersal may be limited by climatic and ecological factors. Thus, further expansion seems unlikely under habitat conditions beyond the current range as experienced in the field experiment. However, to draw conclusions about the potential for further expansion of *C. claviculata*

and expansion rate under ongoing climate change, more detailed studies such as spatial analysis of potential habitats or range models for the entire range border would be necessary.

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2. Vegetation databases as a tool to analyze factors affecting the range expansion of the forest understory herb *Ceratocarpus claviculata*

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Abstract

The eu-atlantic forest herb *Ceratocarpus claviculata* showed a recent increase in frequency within its native range and an east- and northward range expansion in central Europe. To gain deeper understanding of factors affecting the range expansion of the species, we analyzed vegetation relevés at three different scales and asked the following questions: As the species occurs in a wide range of vegetation types, is variation in community composition across the entire range related to climatic environmental zones and tree cover? Are there differences in species composition and habitat characteristics between the native range and the two invaded regions southern Sweden and north-eastern Germany? Did community composition and habitat characteristics within the native range (The Netherlands) change between “before 1970” and “1990–2006”?

Using vegetation plots from the entire distribution range, climatic environmental zones explained 68.9% of the total canonical Eigenvalue. Differences in floristic composition and habitat characteristics between the two invaded regions were as large as between native and invaded range sites. However, relevés from the invaded range were generally characterized by anthropogenic disturbance. Accordingly, abundance of *C. claviculata* was positively linked to silvicultural intensity. New relevés from 1990–2006 were characterized by higher Ellenberg nutrient indicator values, lower species diversity, higher proportions of neophytic and hemerobic species and showed a lower cover of the study species than old relevés from before 1970.

Across the range of *C. claviculata*, climatic environmental zones determine vegetation composition. Accordingly, the species is characterized by a broad macroclimatic amplitude. Vegetation composition and structure differ significantly between the two regions of the new range. Thus, the species has successfully established under various biotic and abiotic environmental conditions. Beyond potential positive effects of soil eutrophication and mild winters, anthropogenic impact may directly facilitate seed dispersal and provide sites and resource conditions suitable for germination and establishment of *C. claviculata*, whereas a decrease of forest management may constrain the species.

Keywords

acidophytic oak-birch forest, clearing, climatic environmental zone, disturbance, Ellenberg indicator value, forest management, global change, habitat requirement, invasibility, therophyte

Nomenclature

Vascular plants (Wisskirchen, Haeupler 1998; for species not included there: Tutin *et al.* 2001), bryophytes (Koperski *et al.* 2000)

Abbreviations

ANOVA = Analysis of Variance

CCA = Canonical Correspondence Analysis

c, s, r = Strategy Types (Grime 1977) c = competitive, s = stress tolerant, r = ruderal

DCA = Detrended Correspondence Analysis

FA = Factor Analysis

IV = Indicator Value

MANCOVA = Multivariate Analysis of Covariance

NEG = North-eastern Germany

NWG = North-western Germany

SD = Standard Deviation

Introduction

Range expansions have been observed in many species during recent decades (e.g. Le Roux and McGeoch 2008). In Central Europe, these may be a trace of ongoing postglacial recolonization processes (Taberlet *et al.* 1998). However, most recent range expansions in cultural landscapes are probably facilitated by human activities such as transport of diaspores, nitrogen inputs, land use changes and disturbance (Chauchard *et al.* 2010), as well as climate change (Walther 2004).

Range expansions imply the establishment of new species in extant communities. This process may be conceptualized as a series of filters selecting the species of a local community from a larger species pool (Integrated Community Concept *sensu* Lortie *et al.* 2004). Successful establishment may be achieved if a new species shows higher fitness than resident species (competitive dominance) or if it occupies a different niche (stabilizing processes) (MacDougall *et al.* 2009). Resource fluctuation (Davis *et al.* 2000) may be an important factor determining establishment success because new species may be able to colonize during periods of low competition through residents, which may occur after disturbance. Thus, the success of local establishment of new species may be driven by a group of features and factors such as (i) species traits, e.g. mode of dispersal, (ii) community characteristics, e.g. vegetation type or resource use by residents, (iii) disturbance, and (iv) abiotic environmental factors such as climate and resource availability (Stohlgren *et al.* 1999, Tilman 2004, Davis *et al.* 2005).

C. claviculata is an annual forest species of the family Papaveraceae (formerly *Fumariaceae*). It grows on humic, acidic soils of the submeridional and temperate zone (Schultze-Motel 1986, Jäger and Werner 2005). Due to its restriction to western Europe, *C. claviculata* is regarded as an eu-oceanic species (Jäger and Werner 2005). However, during the last decades the species showed both an increase in frequency within its range (Buttler 1986, van der Eerden *et al.* 1998, Decocq 2000, Hill *et al.* 2004) and a rapid range expansion east- and northwards into sub-continental and northern-temperate regions (Benkert *et al.* 1995, Oredsson 2005). Currently, it occurs in northern Portugal and north-western Spain, France, Belgium, The Netherlands, north-western Germany, Great Britain, Ireland, Denmark and scattered in southern Norway, southern Sweden and north-eastern Germany. The latter two regions represent the newly invaded range where it was first detected about 25 years ago (Benkert *et al.* 1995, Oredsson 2005).

Several ideas have been proposed to explain the recent spread of *C. claviculata*. These are closely linked to the sequence of factors that determine the invasibility of a local community (Lortie *et al.* 2004, Davis *et al.* 2005). A) Dispersal: since *C. claviculata* lacks structures for long-distance dispersal, anthropogenic activities, such as transport of wood and forest management (clearcutting) are made responsible for the fast regional expansion and local spread, respectively (Buttler 1986, Benkert *et al.* 1995, Decocq 2000, Lethmate *et al.* 2002, Horstmann 2005, Oredsson 2005). B) Increased winter temperatures: after seeds of the species have reached a new locality, mild winter temperatures, potentially a consequence of climate change, may facilitate seedling survival and the establishment of new populations (Folland and Karl 2001, Lethmate *et al.* 2002). C) Nitrogen availability: increased atmospheric nitrogen inputs as a driving factor of floristic changes in forests of central Europe (Kuhn *et al.* 1987, Thimonier *et al.* 1992, van Dobben *et al.* 1999) may increase the cover of this species after successful establishment (Pott and Hüppe 1991, Vannerom *et al.* 1994, van der Eerden *et al.* 1998).

Despite a wealth of knowledge concerning factors that govern plant invasions and range expansions, these still present an apparent paradox (Sax and Brown 2000): why are some organisms, which originate from distant localities and had no chance to adapt to the local environment and the extant communities of the new range, able to establish successfully? In order to gain deeper understanding of factors governing the range expansion of *C. claviculata*, we analyzed vegetation relevés at three different scales: (i) We analysed relevés across the *entire range* extracted from various databases to

quantify the effects of different climatic zones and tree cover on the variation in community composition. Floristic patterns across the entire range were analyzed using an Indicator Species Analysis. (ii) Using own relevés from north-western Germany (native range) and north-eastern Germany and Sweden (invaded range), we tested whether abundance of *C. claviculata*, community composition and habitat characteristics differed between the invaded range and that part of the native range, which was presumably the source for colonization of the new range sites (Benkert *et al.* 1995, Oredsson 2005). (iii) As species has also increased within the native range during the past decades, we tested whether the abundance of *C. claviculata*, community composition and habitat characteristics differed between relevés from the period “before 1970” and “between 1990–2006” using data from the Dutch National Vegetation Database.

Community ecology of the study species

C. claviculata has been described as a constituent of various forest communities in different parts of its range and is considered a character species of the acidophytic oak birch forests of the order *Quercetalia roboris* Tx. 1931. Here, the species has its main occurrence in the associations *Deschampsio flexuosae-Quercetum roboris* Passarge 1966 and the *Betulo pendulae-Quercetum roboris* Tx. 1930 nom. invers. propos. Owing to its eu-atlantic distribution, it is further acknowledged as differential species for western variants of these communities in north-western Germany, France and Benelux (Härdtle *et al.* 1997). In the UK, *C. claviculata* is part of similar acidophytic communities of the *Quercus robur-Pteridium aquilinum-Rubus fruticosus* woodland and the *Quercus* spp.-*Betula* spp.-*Deschampsia flexuosa* woodland (Rodwell 1998). In France and Belgium, the species is found in acidophytic, Atlantic beech forests of the alliance *Ilici-Fagion* Br.-Bl. 1966. In Spain, it grows in hyperoceanic pedunculate oak forests, Orocantabrian north-western Iberian birch-sessile oak forests and Cantabrian-Euskaldian mixed oak-ash forests (Bohn *et al.* 2003). In addition, *C. claviculata* occurs on poor acidic sands in north-eastern Germany in more continental, dry, secondary spruce and pine forests of the alliance *Dicrano polyseti-Pinion sylvestris* (Libbert 1933) W. Matuszkiewicz 1962 nom. cons. propos. in the class *Vaccinio-Piceetea* Br.-Bl. in Br.-Bl. *et al.* 1939 (Berg *et al.* 2001). Within the same class, the species is also found on peat soils of disturbed bogs in secondary birch fen-woods of the *Betuletum pubescentis* Tx. 1937 (Pott 1995, Meyer and Voigtländer 1996).

Outside forests, the species is often found in ecotonal communities. Strongholds of *C. claviculata* occurrences across the entire geographic range are those in disturbed and sun exposed situations of clearings. Within the acidophytic forest clearing communities, stands with *C. claviculata* comprise the association *Corydalis claviculatae-Epilobietum angustifolii* Hülbusch and Tx. 1968 (Berg *et al.* 2001, 2004, Dengler *et al.* 2007). Other ecotonal communities are along forest edges, hedges, roads, ditches, and tracks. These stands have been assigned to different syntaxa within the *Pruno-Rubion radulae* Weber 1974, *Origanetalia vulgaris* Th. Müller 1961, *Convolvulion sepium* Tx. 1947 and *Alliarion* Oberd. (1957) 1962 (Castroviejo 1975, Jäger and Werner 2005, BfN 2008).

Finally, the species occurs in dwarf-shrub heaths and sometimes even in pioneering, xerophilous communities on schist outcrops. In Brittany, these occurrences were attributed to the *Erico cinereae-Vaccinietum myrtilli typicum* (Gloaguen and Touffet 1973) Clément 1978 and the *Festuco-Sedetum anglici* Clément *et al.* 1980 (Clément and Touffet 1978, Clément *et al.* 1980).

Material and Methods

Datasets

Entire range (=dataset 1)

We obtained vegetation relevés containing *C. claviculata* from the entire range, irrespective of habitat type, from national, county and regional databases (denoted by their IDs from the Global Index of Vegetation-Plot Databases (GIVD), Dengler *et al.* 2011): Spain (EU-00-004, Font *et al.* 2010), France (EU-FR-003, Brisse *et al.* 1995), Flanders (EU-BE-001, Vandenbusche and Hoffmann 2001), The Netherlands (EU-NL-001, Schaminée *et al.* 2006), the UK (EU-GB-001, Rodwell 2012), Germany (EU-DE-013, Ewald *et al.* 2010), Mecklenburg-Vorpommern (EU-DE-001, Berg and Dengler 2004), and north-western Germany (EU-DE-010, Pepppler-Lisbach 2012, EU-00-008 and EU-DE-019 Heinken 2012 a,b). These data were complemented with other published (Pollmann and Lethmate 2006) and unpublished (several volunteers) relevés from forests and woodlands. Since the Dutch database contained 4481 relevés with *C. claviculata*, whereas there were e.g. only 45 relevés from Spain, we randomly selected 300 relevés from The Netherlands (all after 1970) to reduce geographical imbalance. However, in an attempt to improve the estimation of community composition and habitat characteristics within the centre of the species distribution range we retained more relevés from the UK, northern Belgium, The Netherlands and north-western Germany in the data set than from adjacent regions. All relevés were additionally filtered according to plausibility (location, species composition) and relevé size. The plot size of the filtered relevés ranged from 4–300 m² (median = 100 m², lower quartile = 100 m², upper quartile = 225 m²), reflecting the heterogeneity of data sets among and within different databases. The final data set contained 762 relevés (Denmark: 2, Flanders: 128, France: 95, Germany: 186, Spain: 45, Sweden: 28, The Netherlands: 231, UK: 47). According to its geographic coordinates, each relevé was assigned to one of five climatic environmental zones defined by Metzger *et al.* (2005): (a) Atlantic North, (b) Atlantic Central, (c) Continental, (d) Lusitanian or (e) Alpine South s.l.; the latter included the original zones Alpine South, Mediterranean Mountains and Mediterranean North. Because relevés of the defined groups varied considerably in mean plot size (ANOVA, $p < 0.001$), we included plot size as covariate in the analyses.

Invaded vs. native range (=dataset 2)

In the invaded range, we selected the northernmost and easternmost regions and visited all currently known localities of the species that contained at least several populations. In the native range, we selected a landscape section of roughly the same dimension as in the invaded range and within the same forest type (conifer dominated stands). Within this section we made random stops at every forest site and selected the first *C. claviculata* population we found.

Invaded and native range differed strongly with respect to continentality (Table 1). However, during the past years, temperature had increased and precipitation decreased which has resulted in warmer winters but drier vegetation periods in all four regions (DWD 2010, SMHI 2010). The invaded range belongs to the Continental zone and was situated in southern Sweden (=S, 12.95–14.63°E, 55.99–56.29°N) and north-eastern Germany (=NEG, 13.96–14.04°E, 52.24–52.30°N); the study sites in the native range belonged to the Atlantic North zone and were situated in north-western Germany (=NWG, 8.22–8.26°E, 52.94–53.05°N).

Substrate type differed only slightly between the three study regions with sand dominating in north-western Germany and north-eastern Germany and sand to silt in southern Sweden. In all regions, the predominating humus types were moder and mor. Mean pH_{KCl} in the upper 10 cm of soil in selected

sites was $3.2 (\pm 0.35 \text{ SD}, n=23)$ in southern Sweden, $3.2 (\pm 0.63 \text{ SD}, n=25)$ in north-western Germany and $3.3 (\pm 0.3 \text{ SD}, n=25)$ in north-eastern Germany (N. Voss unpublished data).

Using the ordinal scale of van der Maarel (1979, 2007), we recorded 73 relevés in the three regions (native range: NWG, $n=25$; invaded range: NEG, $n=25$ and S, $n=23$). The size of the plots varied between 25 and 100 m² (mean=69.3m², lower quartile=36 m², upper quartile=100 m²). Both, a varying size and shape of the plots were chosen to account for differences in the homogeneity of the forest vegetation and the patchy occurrence of the study species. There was, however, no significant difference in relevé size between the three regions (ANOVA, $p=0.090$). However, we also accounted for differences in relevé size in the analysis of this data set (see Statistical analyses).

We estimated the cover of the tree, shrub, herb, and bryophyte layers as well as the proportion of litter and bare soil in the field. The height of the herb layer was estimated to obtain information on vegetation structure. We did this by approximating the height by measuring at the level of main biomass production. The impact of disturbance through silviculture was estimated by assigning an ordinal value (none=0, little=1, much=2) for (a) logging, (b) woodpiles, (c) skid trails and other tracks. The values were summed so that each plot obtained a score between 0 and 6.

Table 1. Average temperature in January (coldest winter month) and from March to June (main growth period of *C. claviculata*) and average precipitation from March to June for the study regions in the native (NWG) and the invaded range (S and NEG) for the past and the present (DWD 2010, SMHI 2010).

Region	Climate station	T _{January} (°C)		T _{March-June} (°C)		Precipitation _{March-June} (mm)	
		1961-1990	2002-2009	1961-1990	2002-2009	1961-1990	2002-2009
S	Kristianstad	-1.3	0.5	8.0	9.0	162	159
NEG	Lindenberg	-1.2	0.2	10.2	11.9	201	156
NWG	Bremen	0.8	2.6	10.0	11.1	230	188

Analysis of temporal variation (=dataset 3)

To compare vegetation composition within the native range between two time periods, we selected relevés from mixed deciduous oak forests from the Dutch National Vegetation Database (ID EU-NL-001, Schaminée *et al.* 2006), which were sampled either in the period 1936–1970 (old relevés) or 1990–2006 (new relevés). All selected relevés contained information on the cover of bryophyte species. To improve homogeneity and comparability of data, old and new relevés were filtered according to the following criteria: (1) presence of *Quercus* spp. in the tree or the shrub layer, (2) cover of coniferous trees <5%, (3) location in the same geographic region, and (4) plot size between 50 and 100m². The final data set contained 181 relevés, 40 from 1936–1970 and 141 from 1990–2006. Plot size did not differ significantly between the two periods (mean₁₉₃₆₋₁₉₇₀ =91.7 m², mean₁₉₉₀₋₂₀₀₆ =92.4m², *t*-test, $p=0.42$, total median=100m², lower quartile=90 m², upper quartile=100m²). Still, we accounted for differences in plot size in the analysis of this data set (see Statistical analyses).

Data management

Relevés were processed using the TURBOVEG software (Hennekens and Schaminée 2001). For datasets i) and iii), species abundances were transformed to ordinal scale (van der Maarel 1979, 2007). In all datasets, species nomenclature was standardized according to the electronic taxonomic reference list GermanSL (Jansen and Dengler 2008); species lacking in this German reference list were added following the nomenclature of Flora Europaea (Tutin *et al.* 2001). Subspecies were subsumed on the species level. Because of the inconsistent determination of species of *Rubus fruticosus* agg. and *Rubus corylifolius* agg., the groups were treated as one taxon *Rubus* sp.

Using the JUICE software (Tichý 2002), the occurrence of a species in the shrub and in the herb layer was merged into one layer. The tree layer was excluded from ordination but was considered in all other analyses as an explanatory variable. To avoid the undue influence of rare species in datasets (ii) and (iii) we removed species that occurred in less than 3% of the relevés. In dataset (i) we deleted only those species that occurred in less than three relevés in order to avoid a loss of too many species from regions with a small number of relevés (e.g. Alpine South or Lusitania with 34 and 22 plots, respectively). Thus, the total number of species was reduced in dataset (i) from 582 to 300, in (ii) from 188 to 81 and in (iii) from 311 to 105 species.

For datasets (ii) and (iii), species richness and Shannon diversity index were calculated in TURBOVEG. Furthermore, using the cover values, we calculated the weighted mean Ellenberg indicator values (Ellenberg *et al.* 1992) for each relevé including all species of the herb and shrub layers with an assigned indicator value. The tree layer was excluded because many of the stands analyzed were forest plantations and thus the tree layer may not reflect the environmental site conditions properly. Including all species, except *C. claviculata*, we further calculated the proportional cover of neophytic and therophytic species and the calibrated proportion of hemerobic species (Frank and Klotz 1990, Klotz and Kühn 2002a) and of ruderal strategy for each relevé (Klotz and Kühn 2002b). Hemerobic species were used as an indicator for human influence on vegetation. The hemerobic level (Klotz and Kühn 2002a) describes the degree of anthropogenic impact on a vegetation unit. Most species of the central European flora have been assigned to one or more of the hemerobic levels defined by Frank and Klotz (1990) and Klotz and Kühn (2002a) and made available in the biological traits database BIOLFLOR (Klotz *et al.* 2002). In order to obtain numeric values for each species, we assigned a value of zero to all species, which were listed as either ahemerobic (“a”) and oligohemerobic (“o”), i.e. species with a chief occurrence in natural vegetation units. Species listed as either “m” (mesohemerobic), “b” (β -euhemerobic), “c” (α -euhemerobic), “p” (polyhemerobic), or “t” (metahemerobic) received a value of one, indicating a higher occurrence and frequency in more human-dominated vegetation units. Species assigned to both groups of hemerobic levels received an intermediate value (e.g. “aomb”=0.5). In order to obtain an indicator for ruderal tendency we proceeded in a similar way with Grime’s (1977) strategy types and assigned a value of one to the r-strategy, whereas c- and s-strategy were assigned a value of zero. Again, for intermediate strategy types proportions of the r-strategy were calculated. Species information for ruderal tendency was also extracted from BIOLFLOR (Klotz and Kühn 2002b).

To obtain percentage cover values for the herb and tree layer in data set (iii), we summed up the percentage cover of the species in the herb and tree layer for each plot, respectively.

Statistical analyses

The analyses were carried out with PC-Ord 5.3 (Indicator Species Analysis; McCune and Grace 2002), CANOCO (DCA, CCA; ter Braak and Šmilauer 2002) and STATISTICA 8.0 (ANOVA, FA, multiple regression, HSD-test, MANCOVA, *t*-test; StatSoft Inc, 2008).

Entire range (=dataset 1)

In order to quantify the effects of different climatic environmental zones and tree cover on the variation in community composition we employed several CCAs for a variance partitioning procedure. We also included plot sizes in the analysis to account for their heterogeneity, which might cause size-dependent ordination patterns (Otýpková and Chytrý 2006). We used log(Area), percentage tree cover and climatic environmental zone (dummy coded) as environmental variables in CCAs. To obtain the effect of a variable, partial CCA was performed controlling for the other two variables (Legendre and Legendre 1998). For all CCAs, significance was tested by permutation tests (1000 permutations). The

ratios of a given canonical Eigenvalue to the sum of all Eigenvalues (total inertia) and to the sum of all canonical Eigenvalues were used to estimate percentages of explained variation of the total inertia and of the total canonical Eigenvalue.

To describe species composition for the different groups, we calculated species constancies and performed Indicator Species Analysis (Dufrêne and Legendre 1997, McCune and Grace 2002). Tree species were considered both in the tree layer as well as in the herb/shrub layer and thus obtained two indicator values in order to include information on the prevailing forest type. Statistical significance of the observed indicator value for a species was evaluated by a Monte Carlo test using 4999 permutations.

Invaded vs. native range (=dataset 2)

In order to visualize the relationships among vegetation plots and to account for the effects of varying plot size in our data we run partial DCA ordination with species cover abundance data. We applied detrending by 2nd order polynomials and used log(Area) as covariate. For interpretation purposes, we overlaid header data such as cover of trees and other derived variables such as proportions of neophytes, therophytes, hemerobic species, ruderal strategy, Ellenberg indicator values and vegetation structure.

As in dataset (1), we calculated constancies and performed Indicator Species Analysis (Dufrêne and Legendre 1997). Tree species were considered both in the tree layer as well as in the herb/shrub layer and thus obtained two indicator values in order to include information on the prevailing forest type. Statistical significance of the observed indicator value for a species was evaluated by a Monte Carlo test using 4999 permutations.

As a result of missing non-species information (e.g. disturbance, height of herbs) in 13 plots, the subsequent statistical analyses were performed using only 60 relevés (NWG, $n=18$; NEG, $n=21$ and S, $n=21$). We carried out MANCOVA to test for significant differences between regions using plot size as covariate (for respective variables see Table 3). Mahalanobis distance was calculated to detect multivariate outliers. To meet the assumptions of normality, the variables cover of shrubs, bryophytes, bare soil and litter as well as proportion of neophytes and hemerobic species were arcsine-square root-transformed and height of herbs was square root-transformed. Cover of *C. claviculata* was first back-transformed to percentage scale (van der Maarel 1979, 2007) and then Box-Cox transformed ($\lambda=-0.922$). Pillai's trace statistic was used because it is most robust to deviations from multivariate normality and the assumption of homogeneity of the variance-covariance matrices across groups with equal sample sizes and approximate univariate normality of response variables (Johnson and Field 1993). Furthermore, we applied HSD tests for unequal group size to assess significant differences of each variable between the regions.

We used stepwise-forward regression in multiple regression to analyze which of the environmental variables (predictors) were significantly associated with the abundance of *C. claviculata* (response variable). As there were many intercorrelated predictors, Factor Analysis (FA, standard varimax axis rotation) was employed for data reduction purposes to avoid problems with multicollinearity (Stevens 2002). FA resulted in five independent, orthogonal factors (Eigenvalue ≥ 1.5). These were interpreted according to the factor loadings of the original predictors (Table 4). Subsequently, the obtained factor scores were used as independent surrogate variables in the multiple regression. Plot size was also included into the model.

Temporal variation (=dataset 3)

As in dataset 2, for visualization and to account for plot size effects we run partial DCA ordination with species cover abundance data. We applied detrending by 2nd order polynomials, used log(Area) as covariate and overlaid header data.

We again calculated constancies and performed Indicator Species Analysis (Dufrêne and Legendre 1997). Tree species were considered both in the tree layer as well as in the herb/shrub layer and thus obtained two indicator values in order to include information on the prevailing forest type. Statistical significance was evaluated by a Monte Carlo test using 4999 permutations.

To test for significant differences between time periods we carried out MANCOVA using plot size as covariate (for respective variables see Table 5). Mahalanobis distance was calculated to detect multivariate outliers. Cover of *C. claviculata* was back-transformed to percentage scale (van der Maarel 1979, 2007) and then power transformed with an exponent of -0.5 to meet assumptions of normality. To assess significant differences of each variable between the time periods, we obtained *p*-values from the univariate results of MANCOVA.

Results

Entire range (=dataset 1)

Taking climatic environmental zones, tree cover and plot size into account as constraining variables in CCA, these explained only 5.9% of total variation in community composition (total inertia=18.4). However, when controlling for the other two variables, each variable yielded a significant effect on floristic composition in partial CCA (Table 2). Climatic environmental zones explained 68.9% of total canonical Eigenvalue (4.1% of total inertia), whereas plot size explained only about 17.6% (1.0% of total inertia) and tree cover even less (8.9% of total canonical Eigenvalue, 0.5% of total inertia). The number of significant indicator species (Appendix S1) with indicator values ≥ 14 for the climatic environmental zones differed strongly with 10 species for the Atlantic-North (among them *Quercus robur* in the tree layer, *Sorbus aucuparia*, *Rubus* sp., *Ilex aquifolium*, *Dryopteris dilatata*, *Frangula alnus*), only four species for the Atlantic-Central (*Quercus rubra*, *Betula pendula*, *Prunus serotina*, *Molinia caerulea*), seven species for the Continental (e.g. *Deschampsia flexuosa*, *Pinus sylvestris*-tree layer, *Impatiens parviflora*, *Rubus idaeus*, *Robinia pseudoacacia* in the tree layer), 12 species for the Lusitanian (e.g. *Epilobium angustifolium*, *Cytisus scoparius*, *Galium saxatile*, *Sambucus racemosa*, *Linaria repens*) and 19 species for the Alpine-South zone, some of which are endemic to the Iberian Peninsula (e.g. *Erica arborea*, *Genista florida*, *Cytisus oromediterraneus*). Across the entire range, the most constant species (mean constancy > 25%) occurring together with *C. claviculata* were *Quercus robur* (37% mean constancy), *Lonicera periclymenum* (27%), *Agrostis capillaris* (26%), *Holcus mollis* (25%) and *Galeopsis tetrahit* (21%).

Table 2. Results of CCA with the explaining variables (Exp. var.) climatic environmental zone (C), log(Area) (A) and percentage cover of trees (T) as well as partial CCAs, each adjusted for two of the variables (Covar.). Canonical Eigenvalues (Eigen.), percentage of explained variance of total inertia (% total inertia), percentage of explained variance of the total canonical Eigenvalue (% can. Eigen.) and *F*-ratio (*F*) for the test of significance of all canonical axes and corresponding probability value (*p*) obtained by the Monte Carlo permutation test (1000 permutations). Total inertia = 18.36.

Exp. var.	Covar.	Eigen.	% Total Inertia	% Can. Eigen.	<i>F</i>	<i>p</i>
C, A, T	-	1.085	5.91	100.0	7.466	0.001
C	A,T	0.747	4.07	68.9	7.709	0.001
A	C,T	0.191	1.04	17.6	7.890	0.001
T	C,A	0.096	0.52	8.9	3.970	0.001

Native vs. invaded range (=dataset 2)

Partial DCA ordination of the species abundances (Fig. 1) yielded a total inertia of 4.5 before, and 4.4 after fitting the covariable log(Area). Thus, plot size accounted for only 1.5% of total inertia.

There was a clear separation of the regions NWG, NEG and S along the first and second axis. Thus, on the one hand, we found floristic differences between native and invaded range sites, but on the other hand there were also large differences between the two invaded regions. The first axis was positively correlated with the cover of shrubs ($r=0.37$), open soil ($r=0.19$, not shown), proportion of neophytes ($r=0.85$), hemerobic species ($r=0.65$), r-strategists ($r=0.50$), therophytes ($r=0.43$, not shown), Ellenberg indicator values for nitrogen ($r=0.56$) and temperature ($r=0.50$), and negatively correlated with Ellenberg indicator value for moisture ($r=-0.49$) and the height of herbs ($r=-0.50$). The second axis was positively correlated with the cover of bryophytes ($r=0.31$) and trees ($r=0.27$) and negatively correlated with litter ($r=-0.40$), herbs ($r=-0.30$) and Ellenberg indicator value for light ($r=-0.34$).

MANCOVA showed significant differences ($p<0.001$) between the three regions in site conditions and habitat characteristics (Table 3).

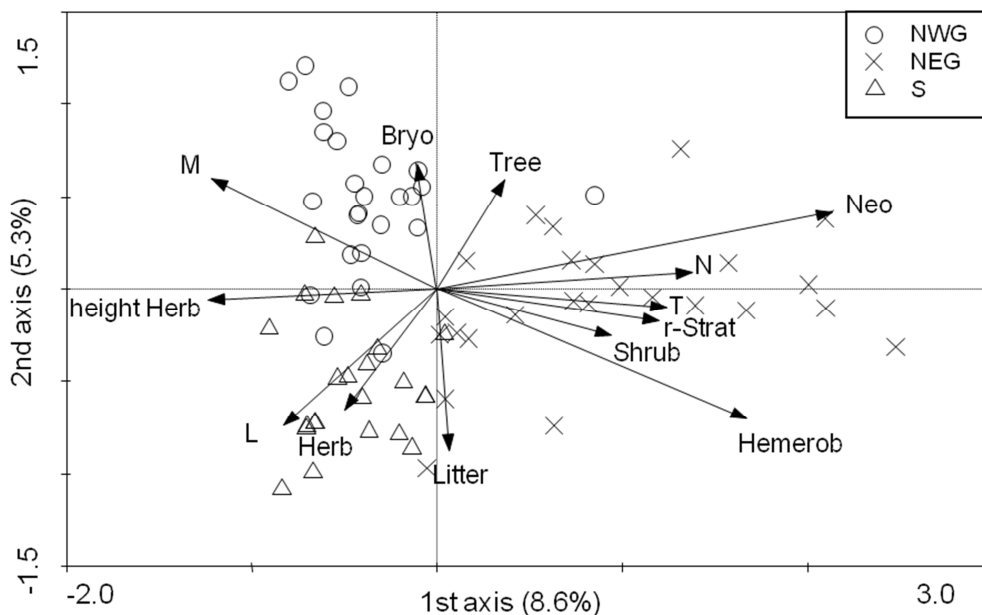


Fig. 1. Partial DCA of native (NWG, $n=25$) and invaded (S, $n=23$ and NEG, $n=25$) range sites adjusted for the covariable plot size. Total inertia=4.38, cumulative explained variance=22%. Only the first two axes were plotted (explained variance=8.6% and 5.3%, respectively). Vectors ($-0.25 \leq r \leq +0.25$) indicate cover sum of litter (Litter), bryophytes (Bryo), herbs (Herb), shrubs (Shrub) and trees (Tree), proportions of hemerobic species (Hemerob), neophytes (Neo) and r-strategists (rStra), Ellenberg indicator values for light (L), moisture (M) nutrients (N) and temperature (T), and the height of the herb layer (height Herb). The vector proportions of therophytes ($r=0.43$, not shown) goes parallel with proportions of r-strategists.

The comparison showed only few consistent differences between native and invaded range. In contrast, we often found either clinal variation from NWG over NEG to S or differences between the two German regions on the one hand and S on the other hand. The covariable plot size was not significant ($p=0.251$). The cover of *C. claviculata* was higher in the invaded range, increasing slightly from NWG to NEG and being significantly higher in S (HSD test, Table 3). Likewise, litter cover, proportions of hemerobic species, therophytes and r-strategists as well as disturbance by forestry and tracks tended to be higher in invaded range sites, whereas the cover of bryophytes tended to be lower.

Indicator Species Analyses (Appendix S2) was consistent with these results featuring nitrophilous species such as *Sambucus nigra*, *Moehringia trinerva* and *Chelidonium majus* and neophytic species such as *P. serotina*, *R. pseudoacacia* and *I. parviflora* in NEG, indicators for moisture, such as

F. alnus and *M. caerulea* in NWG and species characteristic for clearings like *S. racemosa*, *R. idaeus*, *D. flexuosa* and *E. angustifolium* in S. The last findings were reflected by a higher proportion of light demanding species in S.

The multiple regression on the cover of *C. claviculata* with the five surrogate predictor variables (from Factor Analysis, Table 4) showed a significant relationship with two factors ($r^2_{\text{corrected}}=0.30$, $df=2$, $F=13.62$, $n=60$, $p<0.001$). Only factor 2 (surrogate: cover of bryophytes and litter) and factor 3 (surrogate: disturbance) correlated significantly with the cover of *C. claviculata*.

Table 3. Habitat characteristics and cover of *C. claviculata* (mean \pm SD) in the native vs. invaded range: north-western Germany (NW-G, native range, $n=18$), southern Sweden (S-S, invaded range, $n=21$) and north-eastern Germany (NE-G, invaded range, $n=21$). Letters indicate significant differences according to HSD test, ($p < 0.05$). MANCOVA was significant for the three regions with Pillai's trace=1.593, $F_{34, 82}=9.45$, $p < 0.001$ but not significant for the covariable plot size (log (Area)) with Pillai's trace=0.353, $F_{17, 40}=1.28$, $p=0.251$. Pearson's regression coefficient is shown for significant relationships ($p < 0.05$) between variables and plot size.

	Native Range		Invaded Range		NEG		Pearson's r (log Area)
	NWG		S				
Cover sum (%):							
Tree	49.2 ^a	± 21.7	27.9 ^b	± 28.8	42.4 ^{ab}	± 16.6	
Shrub	7.2 ^a	± 6.4	10.5 ^a	± 17.9	22.8 ^b	± 17.5	
Herb	43.3 ^a	± 19.6	45.7 ^a	± 21.7	33.1 ^a	± 17.6	
Moss	38.1 ^a	± 32.2	5.1 ^b	± 6.5	28.8 ^a	± 24.2	
Litter	61.8 ^a	± 32.2	93.5 ^b	± 6.7	69.8 ^a	± 23.7	
Bare soil	0.0 ^a	± 0.0	1.3 ^a	± 2.6	1.5 ^a	± 4.5	
<i>C. claviculata</i>	4.4 ^a	± 1.7	9.5 ^b	± 6.5	6.8 ^{ab}	± 8.4	
Height herbs (cm)	53.4 ^a	± 23.4	63.8 ^a	± 36.9	23.3 ^b	± 9.3	
Ellenberg IV							
Light	5.83 ^{ab}	± 0.28	6.01 ^b	± 0.41	5.67 ^a	± 0.32	
Moisture	5.37 ^a	± 0.31	5.18 ^a	± 0.36	4.78 ^b	± 0.25	
Nitrogen	4.94 ^a	± 0.69	5.05 ^a	± 0.66	5.69 ^b	± 0.77	-0.24
Temperature	4.77 ^a	± 0.55	4.99 ^{ab}	± 0.46	5.30 ^b	± 0.45	
Disturbance	2.27 ^a	± 1.23	4.76 ^b	± 1.92	3.14 ^a	± 1.53	
Proportion cover of:							
Neophytes	0.044 ^b	± 0.038	0.007 ^a	± 0.022	0.185 ^c	± 0.110	
Hemerobic species	0.254 ^a	± 0.101	0.377 ^b	± 0.157	0.504 ^c	± 0.127	
Therophytes	0.045 ^a	± 0.055	0.055 ^{ab}	± 0.071	0.098 ^b	± 0.071	-0.30
r-Strategists	0.039 ^a	± 0.038	0.053 ^{ab}	± 0.039	0.080 ^b	± 0.049	-0.38

Comparison of old and new relevés (=dataset 3)

The partial DCA yielded a total inertia of 5.5 after fitting the covariable plot size (log[Area]). Without adjustment, total inertia was 5.6; thus, plot size accounted for only 1.9% of total inertia (Fig. 2). Using grouping according to time period, a weak separation of plots from before 1970 and after 1990 was evident along the second axis (more pronounced on the plain of second and third axis). Vectors for Shannon index ($r=0.15$, but -0.54 for third axis), cover of herbs ($r=0.24$) and trees ($r=0.13$), Ellenberg indicator values for temperature ($r=0.33$), reaction ($r=0.69$) and nutrients ($r=0.65$), cover of herbs ($r=0.24$ but with third axis -0.55), proportion of therophytes ($r=0.32$) and r-strategists ($r=0.59$) and hemerobic species ($r=0.59$) were correlated with the first axis. Ellenberg indicator values for light

($r = -0.61$) and for moisture ($r = -0.31$) and proportion of neophytes ($r = -0.22$) were correlated with the second axis.

Habitat characteristics differed significantly between the two time periods and were in part significantly correlated with plot size (Table 5). Cover of *C. claviculata* was higher in plots from before 1970 than in plots after 1990. Shannon diversity was lower in the newer relevés. This corresponds to a more than eight-fold higher number of indicator species (Appendix S3) for old plots. Furthermore, Ellenberg indicator values for light, temperature, moisture, and nutrients were slightly higher in current than in old plots. Also, the proportion of hemerobic species and neophytes was larger in the new relevés, as indicated by species such as *P. serotina*, *Amelanchier lamarckii* and *Q. rubra*.

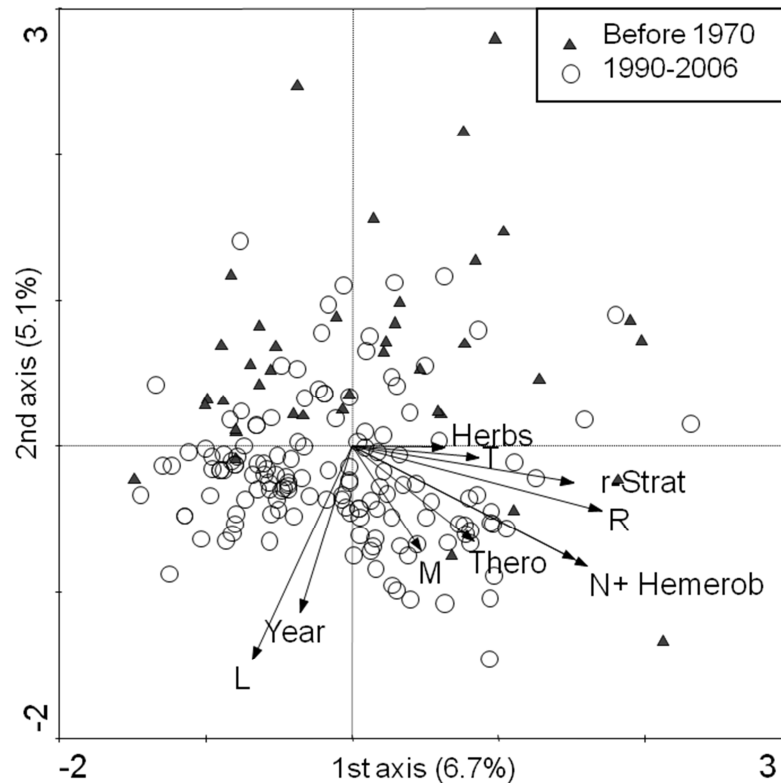


Fig. 2. Partial DCA of relevés from old (before 1970, $n=40$) and new (1990–2006, $n=141$) Dutch relevés adjusted for the covariable plot size. Total inertia=5.52, cumulative explained variance=18.4%. Only the first two axes were plotted (explained variance=6.7 and 5.1, respectively). Vectors ($-0.25 \leq r \leq +0.25$) indicate cover sum of herbs (Herbs), proportions of hemerobic species (Hemerob), r-strategists (r-Strat), and therophytes (Thero) and Ellenberg indicator values for light (L), moisture (M) nutrients (N) and temperature (T).

Discussion

Entire range

The CCA yielded only a very low percentage of variance explained by the environmental variables used. Explained variance depends on the number of species, plots and further unknown environmental factors (we included only two in the ordination). Thus, rather than the ratio of explained to unexplained variance, the percentage of explained variance of the total canonical eigenvalue of each variable should be considered (Leyer and Wesche 2007). Despite the low number of variables included, our results reflect quite well the underlying patterns of vegetation composition and show the appropriateness of using the climatic environmental classification of Metzger *et al.* (2005) for interpretation of species composition. However, results of large datasets from various databases from across the entire range of a species should be interpreted with caution. Because the original authors usually applied various sampling techniques for various study aims, huge heterogeneity in the

resulting dataset might be either associated with actual environmental heterogeneities or/and with differences in sampling method/intention. The latter was shown by the wide range of plot sizes which accounted for a low proportion of total variance but, explained 17.6% of canonical variation.

In total, 68.9% of the explained variation resulted from the effect of the climatic environmental zones after accounting for the effects of tree cover and plot size. The climatic environmental zones largely depend on minimum and maximum temperature of the months January, April, July, and October, on precipitation during these months and on oceanity. As expected, climatic stratification accounts for a large part of species composition. Relevés originating from the Atlantic Central zone yielded only a low number of significant indicator species, which indicates a large floristic heterogeneity.

Table 4. Relationship between *C. claviculata* and environmental variables. a) Factor loadings, Eigenvalues and explained variance of Factor Analysis, $n=60$. The factors scores were used as independent surrogate variables in multiple regression for variables with loadings > 0.700 or < -0.700 (in bold). Variables transformed before analysis are labeled with ^t. b) Results from stepwise-forward GRM with the five surrogate variables and plot size as predictors for the cover of *C. claviculata* (%). Only significant relationships ($p < 0.05$) are shown. Area was not significant. SS=sum of squares, df=degrees of freedom, $F=F$ -ratio, p =error probability, β =regression coefficient, EV=explained variance.

a)					
Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Cover sum (%)					
Tree	0.208	0.113	0.619	-0.257	-0.112
Shrub ^t	0.343	-0.154	0.444	0.196	0.401
Herb	-0.373	0.058	-0.065	0.104	0.730
Bryophyte ^t	0.056	-0.964	0.061	-0.085	0.022
Litter ^t	-0.027	0.957	-0.031	0.005	0.049
Bare soil ^t	-0.190	0.224	-0.103	0.534	-0.496
Height herbs (cm) ^t	-0.763	0.132	0.025	-0.035	0.280
Disturbance					
Tracks	-0.393	-0.036	0.566	-0.153	0.175
Skid trails	-0.150	0.110	-0.745	0.048	-0.119
Logging	0.026	0.072	-0.707	-0.145	0.300
Ellenberg IV					
Light	-0.296	0.207	-0.117	-0.282	0.555
Temperature	0.440	0.528	0.017	0.381	-0.012
Moisture	-0.656	-0.173	0.091	0.029	-0.022
Nutrients	0.453	0.230	0.162	0.478	0.044
Proportion					
Neophytes ^t	0.743	-0.118	0.262	0.365	-0.140
Therophytes	0.114	-0.053	-0.035	0.867	0.035
r-Strategists	0.099	0.128	-0.042	0.919	-0.058
Hemerobic species ^t	0.444	0.447	0.102	0.521	0.135
Eigenvalue	2.789	2.608	2.106	2.912	1.521
Proportion explained variance	0.155	0.145	0.117	0.162	0.084

b)

Effect	SS	df	F	P	β	EV (%)
Intercept	39.72	1	3415.68	<0.001		
Factor 2 (Cover of litter/bryophytes)	0.05	1	4.20	0.045	0.223	5.1
Factor 3 (Disturbance)	0.27	1	23.03	<0.001	-0.523	28.0
Error	0.66	57				

The Atlantic-Central zone is the largest of the climatic geographic regions and thus a variety of environmental habitat conditions are included within this zone. In addition, the low number of indicator species might be due to the literal “central position”: the large Atlantic Central zone is the centre of the range of many typical elements of the European flora and thus shares many of these species with the adjoining zones. In contrast, the Alpine South zone with relevés largely originating from Spain, because of its geographic isolation, showed the most differing species composition.

Table 5. Comparison of old (before 1970, $n=40$) and new Dutch relevés (1990–2006, $n=141$). Mean \pm SD of habitat characteristics and cover of *C. claviculata* of the two time periods (p values from ANOVA) and Pearson’s correlation between the environmental variables and the plot size (Area) (only shown if $p < 0.05$). Time period (Pillai’s trace=0.3875, $F_{13, 166}=8.126$, $p < 0.001$) and the covariate “Area” (Pillai’s trace=0.209, $F_{13, 166}=3.383$, $p < 0.001$) are significant in MANCOVA.

Variable	before 1970	1990–2006	p	Pearson’s r (Area)
Cover sum (%)				
Tree	60.7 \pm 41.6	64.2 \pm 29.2	0.538	
Herbs	296.2 \pm 95.1	282.9 \pm 131.6	0.552	-0.26
<i>C. claviculata</i>	6.1 \pm 8.0	3.5 \pm 5.5	0.010	
Shannon Index	3.16 \pm 0.36	2.79 \pm 0.42	<0.001	-0.25
Ellenberg indicator values				
Light	5.35 \pm 0.44	5.65 \pm 0.42	<0.001	
Temperature	5.05 \pm 0.44	5.20 \pm 0.43	0.049	
Moisture	5.35 \pm 0.47	5.55 \pm 0.48	0.020	-0.21
Reaction	3.73 \pm 0.82	3.69 \pm 0.73	0.801	-0.33
Nitrogen	4.15 \pm 0.89	4.79 \pm 0.92	<0.001	-0.27
Proportion of:				
Neophytes	0.012 \pm 0.026	0.046 \pm 0.045	<0.001	
Therophytes	0.050 \pm 0.037	0.043 \pm 0.053	0.485	-0.18
r-Strategists	0.059 \pm 0.037	0.048 \pm 0.039	0.098	-0.27
Hemerobic species	0.419 \pm 0.090	0.461 \pm 0.098	0.018	-0.21

Native vs. invaded range

The two study regions in the invaded range exhibited differences in vegetation composition as large as those between the native and invaded range. These results suggest that *C. claviculata* has successfully established in various vegetation types that differ among the three regions. However, there were no systematic differences in habitat characteristics between native and invaded range sites.

We found a higher mean cover of *C. claviculata* in the invaded range. This is surprising as the invaded regions are, despite milder winters during the past decade, still characterized by continental climate. Furthermore, in all regions many individuals were observed which germinated apparently in autumn and survived despite low winter temperatures, even after the long and cold winters in 2008 and 2009. These data and observations suggest that microclimatic conditions, which may be affected by the

surrounding vegetation and litter, might be more important for germination, establishment and survival than macroclimate (e.g. Buttler 1986, Voss unpublished data). This is also in line with the positive impact of litter cover on *C. claviculata* abundance as revealed by the multiple regression.

The results of the multiple regression also suggested that abundance of *C. claviculata* was associated with disturbance through silviculture, which, in fact, was higher in the new range. A larger human impact in the new range might also be reflected by a higher proportion of hemerobic and ruderal species, indicating a higher availability of microsites for the establishment of short-lived species, and by higher proportions of neophytic and nitrophilous species (Lososová *et al.* 2006, Chytrý *et al.* 2008). These results are consistent with the novel niche theory (MacDougall *et al.* 2009), which claims that disturbed habitats, especially those altered by human activities, are often successfully colonized by new species because they represent novel conditions for residents. Many species entering disturbed habitats have weed-like traits, such as the annual, self-pollinating *C. claviculata*. It was suggested that the often-observed higher abundance of introduced species in their new range results from a higher rate of disturbance, which creates colonization gaps with higher substrate availability and low competition (Pickett and White 1985, Davis *et al.* 2000, Chmura and Sierka 2007, Fausch 2008).

In addition to the present impact of silviculture in conifer-dominated forests other past or recent disturbances may have promoted *C. claviculata*. Factors such as wild boars, game passes, and human leisure activities may have positively affected both the dispersal of *C. claviculata* and the disturbance of forest sites (Buttler 1986, Pott 1995, Dzwonko and Loster 1997). In fact, recently L. Tandler and C. Peppeler-Lisbach (unpubl. results) showed that the occurrence of *C. claviculata* was correlated with the presence of forest roads or paths.

The question is prompted: how could a species like *C. claviculata*, which has no adaptation to long distance dispersal, overcome large distances of tens or hundreds of kilometers prior to its successful establishment in the new range? Occurrences might be related to haul roads and/or seeds or fruits attached to timber bark that may be translocated through forestry machinery or shipping of wood (Buckley *et al.* 2003). Large amounts of pulpwood and sawtimber were transported to Swedish sawmills and paper plants after severe storms in north-western Germany and The Netherlands in 1972. These transports most probably facilitated the introduction of *C. claviculata* to Sweden (Oredsson 2005). Moreover, expansion in the new range might be related to an expansion within the native range. An increased propagule pressure in the native range caused e.g. by more favorable nutrient supply or climate, may result in an increased dispersal probability and thus provides a further explanation for successful expansion.

However, our results suggest that beyond the proposed effects of increased nutrient availability and mild winter temperatures (Pott and Hüppe 1991, Vannerom *et al.* 1994, van der Eerden *et al.* 1998, Lethmate *et al.* 2002), anthropogenic impact may directly facilitate seed dispersal and provide sites and resource conditions suitable for germination and establishment of *C. claviculata*.

Comparison of old and new Dutch relevés

Plots from deciduous oak forests from before 1970 and after 1990 differed with respect to community composition and species diversity. Cover of *C. claviculata* decreased significantly between the two time periods despite an increase of Ellenberg indicator values for nutrients and an increased anthropogenic impact on the studied forest community, as indicated by an increase of neophytic and hemerobic species. In contrast, van Dobben *et al.* (1994) found that the cover of *C. claviculata* increased in The Netherlands by 17% between 1984 and 1993 in parallel with strong changes of understory species composition probably owing to increased ammonia deposition (van Dobben *et al.* 1994). However, in contrast to our data from acidophytic oak forests, van Dobben *et al.* (1994) focused on pine forests, which are more intensively managed than deciduous forests. Furthermore,

traditional deciduous woodland management techniques (featuring coppicing, canopy opening, litter removal, grazing, natural treefalls and soil disturbance), which favored germination and establishment of ruderal and therophytic species, have largely been abandoned (Brunet *et al.* 1996, Förster 1998, Rodwell 1998). The demise of traditional management techniques and conversion from more open canopies to dense timber forests caused a general decline in herb cover, which in turn may have negative impacts on therophytic and ruderal species (Brunet *et al.* 1997, Diekmann *et al.* 1999, Strandberg *et al.* 2005, Gondard *et al.* 2007, Spitzer *et al.* 2008). Hence, lacking traditional management and disturbance may have caused the observed decrease of *C. claviculata* and Shannon index in oak forests, which are considered as the species' main habitat in its north-west European range (Härdtle *et al.* 1997). As we used data from vegetation databases, the relevés of the two time periods compared did not come from identical locations, which would have been the case in a permanent plot study. Although the database approach is valuable for temporal comparisons in a given vegetation type (Wildi 1989, Holeksa and Wozniak 2005), results should be interpreted with caution. Observed changes might result from sampling bias, if e.g. relevés from before 1970 were from ancient oak forest whereas the younger relevés originated from younger stands. Land-use history data might be useful to solve this problem but were not available in this dataset. However, we tried to reduce potential sampling bias by using several selection criteria for the extraction of relevés from the database.

Conclusions

Our analyses suggest that *C. claviculata* occupies a relatively broad ecological niche as it occurs in a wide range of vegetation types. Across the entire range, macroclimatic conditions are a significant driver of vegetation composition. Vegetation composition and structure differ significantly between the two regions of the new range, indicating that the species has successfully established under different biotic and abiotic environmental conditions. However, our analyses suggest that in general, anthropogenic disturbance of resident vegetation and soil surface play an important role for local dispersal and population establishment by providing sites and suitable conditions for germination of *C. claviculata*. In contrast, for habitats in open oak-birch forests, our temporal comparison showed rather a decrease of abundance of *C. claviculata*. This decrease might reflect changes in forest management during the past century that resulted in a closure of tree canopies in recent forests.

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Appendix

S1. Significant indicator species ($IV \geq 14$; $p_{IV} < 0.05$) for the respective climatic environmental zone and constancies for each of the five zones representing the total range of *C. claviculata*. Below: Species which are common for all regions (constancy > 10% in four of five groups). Species of the tree layer are indicated by “tree”.

		Indicator value	Constancy			
		Continental <i>n</i> = 92	Atl. North <i>n</i> = 248	Atl. Central <i>n</i> = 366	Lusitanian <i>n</i> = 22	Alpine South <i>n</i> = 34
Continental						
<i>Deschampsia flexuosa</i>	29.2	86	48	50	59	44
<i>Pinus sylvestris</i> , tree	27.9	58	23	28	0	12
<i>Impatiens parviflora</i>	20.3	23	2	1	0	0
<i>Rubus idaeus</i>	18.8	54	48	10	45	12
<i>Arrhenaterum elatior</i>	16.5	27	1	1	0	21
<i>Robinia pseudoacacia</i> , tree	15.1	15	0	0	0	0
<i>Robinia pseudoacacia</i>	14.0	14	0	0	0	0
Atlantic North						
<i>Quercus robur</i> , tree	32.0	9	57	32	27	3
<i>Sorbus aucuparia</i>	28.3	66	75	51	32	3
<i>Fagus sylvatica</i>	25.7	14	35	6	0	0
<i>Rubus spec.</i>	24.9	42	67	47	5	18
<i>Ilex aquifolium</i>	24.4	0	33	10	0	3
<i>Dryopteris dilatata</i>	22.6	17	57	44	9	21
<i>Frangula alnus</i>	18.6	32	48	47	5	6
<i>Maianthemum bifolium</i>	18.4	9	27	3	0	0
<i>Dryopteris carthusiana</i>	17.6	49	47	34	9	3
<i>Deschampsia cespitosa</i>	15.3	4	19	1	0	0
Atlantic Central						
<i>Prunus serotina</i>	17.5	27	27	40	0	0
<i>Betula pendula</i>	14.9	15	11	26	0	0
<i>Molinia caerulea</i>	14.5	13	36	34	14	0
<i>Quercus rubra</i>	13.9	3	4	19	0	0
Lusitanian						
<i>Epilobium angustifolium</i>	29.0	17	17	9	45	0
<i>Cytisus scoparius</i>	22.8	5	2	10	41	12
<i>Cirsium palustre</i>	21.0	2	3	3	27	0
<i>Galium saxatile</i>	20.9	13	16	10	45	15
<i>Salix caprea</i> , tree	17.1	0	0	1	18	0
<i>Lotus pedunculatus</i>	17.0	0	0	1	18	0
<i>Salix cinerea</i>	16.3	1	2	3	23	0
<i>Senecio sylvaticus</i>	16.2	0	8	8	23	0
<i>Pseudotsuga menziesii</i>	16.1	1	3	4	23	0
<i>Silene vulgaris</i>	16.1	0	0	2	18	0
<i>Genista pilosa</i>	15.7	0	0	2	18	0
<i>Sambucus racemosa</i>	14.0	24	2	2	27	0
Alpine South						
<i>Erica arborea</i>	41.1	0	0	0	23	53
<i>Teucrium scorodonia</i>	34.4	0	3	22	23	56

	Indicator value	Constancy				
		Continental	Atl. North	Atl. Central	Lusitanian	Alpine South
		<i>n</i> = 92	<i>n</i> = 248	<i>n</i> = 366	<i>n</i> = 22	<i>n</i> = 34
<i>Pteridium aquilinum</i>	33.4	1	20	25	27	74
<i>Stellaria holostea</i>	31.1	7	27	7	32	62
<i>Arenaria montana</i>	29.4	0	0	0	0	29
<i>Genista florida</i>	28.3	0	0	0	9	32
<i>Dryopteris filix-femina</i>	25.7	8	5	5	18	41
<i>Conopodium majus</i>	25.5	0	0	1	0	26
<i>Poa nemoralis</i>	22.8	5	1	2	5	32
<i>Viola riviniana</i>	20.9	0	2	1	18	32
<i>Quercus petraea</i> , tree	20.0	2	3	2	0	26
<i>Asphodelus albus</i>	18.8	0	0	0	5	21
<i>Ilex aquifolium</i> , tree	17.9	0	1	0	36	32
<i>Vaccinium myrtillus</i>	17.8	10	38	17	18	47
<i>Euphorbia amygdaloides</i>	15.4	0	0	0	5	18
<i>Fagus sylvatica</i> , tree	15.2	1	29	4	23	32
<i>Luzula sylvatica</i>	14.9	11	2	1	9	24
<i>Cytisus oromediterraneus</i>	14.7	0	0	0	0	15
<i>Saxifraga spathularis</i>	14.0	0	0	0	18	24

Common species	Constancy				
	Continental	Atl. North	Atl. Central	Lusitanian	Alpine South
<i>Quercus robur</i>	58	55	55	14	3
<i>Lonicera periclymenum</i>	14	44	25	18	32
<i>Agrostis capillaris</i>	38	16	24	41	9
<i>Holcus mollis</i>	9	23	27	36	29
<i>Galeopsis tetrahit</i>	21	33	23	27	3
<i>Corylus avellana</i>	11	24	9	23	18
<i>Betula pendula</i> , tree	12	11	12	27	0

S2. Indicator species (for $p_{IV} < 0.05$) of the native (NW Germany) and the invaded range (S Sweden; NE Germany). Below: Species which are common to all regions (constancy of each of the groups $> 30\%$). Species of the tree layer are indicated by “tree”.

	Indicator value	Constancy		
		NW Germany	S Sweden	NE Germany
		<i>n</i> = 25	<i>n</i> = 23	<i>n</i> = 25
NW Germany				
<i>Dryopteris dilatata</i>	50.2	68	26	8
<i>Frangula alnus</i>	42.3	72	26	28
<i>Galium aparine</i>	39.7	44	0	8
<i>Eurhynchium praelongum</i>	31.2	48	4	28
<i>Molinia caerulea</i>	29.9	44	4	16
<i>Betula pubescens</i> , tree	26.2	36	13	0
<i>Quercus robur</i> , tree	26.2	36	0	12
<i>Hypnum cupressiforme</i>	26.0	52	22	32
<i>Lonicera periclymenum</i>	20.7	28	4	4
<i>Eurhynchium striatum</i>	16.7	20	0	4
<i>Pseudotsuga menziesii</i> , tree	16.0	16	0	0
S Sweden				
<i>Sambucus racemosa</i>	50.7	0	57	16
<i>Rubus idaeus</i>	46.7	76	83	12
<i>Betula pubescens</i>	39.9	20	52	0
<i>Deschampsia flexuosa</i>	39.8	80	100	84
<i>Luzula sylvatica</i>	34.8	0	35	0
<i>Epilobium angustifolium</i>	27.4	28	43	4
<i>Maianthemum bifolium</i>	21.7	0	22	0
<i>Picea abies</i>	17.4	0	17	0
<i>Poa trivialis</i>	16.2	8	22	0
NE Germany				
<i>Prunus serotina</i>	65.2	48	0	84
<i>Robinia pseudoacacia</i>	52.0	0	0	52
<i>Robinia pseudoacacia</i> , tree	48.0	0	0	48
<i>Impatiens parviflora</i>	45.5	8	9	60
<i>Arrhenatherum elatius</i>	40.6	0	13	56
<i>Sambucus nigra</i>	40.0	4	0	44
<i>Acer platanoides</i>	32.0	0	0	32
<i>Calamagrostis epigejos</i>	28.0	0	0	28
<i>Moehringia trinervia</i>	26.1	0	13	36
<i>Chelidonium majus</i>	25.0	4	0	32
<i>Betula pendula</i> , tree	21.0	4	0	24
<i>Betula pendula</i>	19.3	4	4	24
<i>Convolvulus arvensis</i>	16.0	0	0	16
<i>Pinus sylvestris</i>	16.0	0	0	16
<i>Poa nemoralis</i>	16.0	0	0	16
<i>Acer platanoides</i>	15.4	4	0	20

Common species	Constancy		
	NW Germany	S Sweden	NE Germany
<i>Pinus sylvestris</i> , tree	60	48	76
<i>Brachythecium rutabulum</i>	64	35	68
<i>Scleropodium purum</i>	40	61	68
<i>Quercus robur</i>	60	43	60
<i>Pleurozium schreberi</i>	48	43	48
<i>Dryopteris carthusianum</i>	56	52	36

S3. Indicator species ($IV \geq 14$; $p_{IV} < 0.05$) and respective constancies of old (before 1970) and recent vegetation relevés (1990–2006). Below: Species which are common for both periods (constancy of each of the groups $> 30\%$). Species of the tree layer are indicated by “tree”.

	Indicator value	Constancy	
		Before1970	Period 1990–2006
		<i>n</i> = 40	<i>n</i> = 141
Before 1970			
<i>Maianthemum bifolium</i>	47.7	65	23
<i>Lonicera periclymenum</i>	47.0	75	48
<i>Frangula alnus</i>	43.4	70	50
<i>Ilex aquifolium</i>	40.6	48	18
<i>Stellaria holostea</i>	39.0	50	16
<i>Vaccinium myrtillus</i>	37.7	50	18
<i>Hedera helix</i>	36.6	48	17
<i>Pteridium aquilinum</i>	33.4	45	11
<i>Molinia caerulea</i>	31.7	48	30
<i>Oxalis acetosella</i>	30.6	38	7
<i>Hieracium laevigatum</i>	28.5	33	4
<i>Luzula pilosa</i>	27.5	28	0
<i>Trientalis europaea</i>	27.5	28	0
<i>Carex pilulifera</i>	25.3	35	14
<i>Melampyrum pratense</i>	24.8	35	9
<i>Dryopteris carthusiana</i>	24.6	35	24
<i>Plagiothecium denticulatum</i>	23.4	25	3
<i>Vaccinium vitis-idaea</i>	22.0	23	1
<i>Scleropodium purum</i>	21.9	28	6
<i>Milium effusum</i>	21.6	23	1
<i>Polypodium vulgare</i>	20.9	25	4
<i>Corylus avellana</i>	20.1	28	9
<i>Viola riviniana</i>	19.4	20	1
<i>Polytrichum formosum</i>	18.9	30	16
<i>Convallaria majalis</i>	18.1	20	2
<i>Rubus idaeus</i>	15.8	23	9
<i>Aulacomnium androgynum</i>	15.8	18	2
<i>Galium saxatile</i>	15.7	23	11
<i>Moehringia trinervia</i>	15.3	18	5
<i>Solidago virgaurea</i>	15.0	15	0
<i>Populus tremula</i>	14.7	20	7
<i>Galium aparine</i>	14.6	20	9
<i>Sorbus aucuparia</i> , tree	14.5	20	11
Period 1990–2006			
<i>Prunus serotina</i>	42.9	15	55
<i>Dryopteris dilatata</i>	33.0	13	40
<i>Amelanchier lamarckii</i>	25.4	5	30
<i>Quercus rubra</i>	14.2	0	14

Common species	Constancy	
	Before 1970	Period 1990–2006
<i>Sorbus aucuparia</i>	86	90
<i>Quercus robur</i> , tree	91	75
<i>Rubus</i> spec.	85	75
<i>Quercus robur</i>	66	58
<i>Deschampsia flexuosa</i>	60	52
<i>Mnium hornum</i>	45	50
<i>Holcus mollis</i>	49	43
<i>Polygonatum multiflorum</i>	30	34
<i>Hypnum cupressiforme</i>	30	32

3. Biological Flora of Central Europe: *Ceratocapnos claviculata* (L.) Lidén

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Abstract

The eu-oceanic therophytic woodland herb *Ceratocapnos claviculata* has been expanding north- and eastwards into north temperate and subcontinental regions during the past decades. The rapid range expansion of the species may be an example of a species which is strongly profiting from global change. Against this background, in the present paper we review the taxonomy, morphology, distribution, habitat requirements, life cycle and biology of the species.

Keywords

Corydalis claviculata, Papaveraceae, Fumariaceae, Plant traits, Range expansion, Species biology, Therophytic woodland plant

Introduction

The annual forest herb *Ceratocapnos claviculata* (*C. claviculata*) has been regarded an eu-oceanic species due to its distribution pattern in western Europe (Jäger and Werner 2005). However, during the last decades the species showed both an increase in frequency within its range (Buttler 1986, Decocq 2000, Hill *et al.* 2004, van der Eerden *et al.* 1998, chapter 2) and a rapid range expansion east- and northwards into sub-oceanic and northern-temperate regions (Benkert *et al.* 1995, Oredsson 2005). Several ideas have been put forward as explanations for the recent spread of *C. claviculata*. These are closely related to the sequence of factors and filters that determine the invasibility of a local community (Lortie *et al.* 2004, Davis *et al.* 2005). 1) Seed dispersal: anthropogenic activities, such as transport of wood and forest management (seed transport through machinery) may be responsible for the fast regional expansion and local spread, respectively (Benkert *et al.* 1995, Buttler 1986, Decocq 2000, Horstmann 2005, Lethmate *et al.* 2002, Oredsson 2005). 2) Increased winter temperatures: after seeds have been dispersed to a new locality, mild winter temperatures (as a consequence of e.g. climate change) may facilitate seedling survival and the establishment of populations (Lethmate *et al.* 2002, Folland and Karl 2001). 3) Soil eutrophication: increased atmospheric nitrogen inputs may increase the performance of this species after successful establishment (Pott and Hüppe 1991, Vannerom *et al.* 1994, van der Eerden *et al.* 1998). Thus, its ongoing range expansion may be another example of a “footprint of climate change” (e.g. Walther *et al.* 2005).

In order to gain deeper understanding of factors governing the range expansion of *C. claviculata*, it appears appropriate to summarize the available information on the biology of *C. claviculata* in a comprehensive review.

The taxonomy and nomenclature follows Wisskirchen and Haeupler (1998).

Taxonomy and morphology

Taxonomy

C. claviculata (L.) Lidén, Anal. Jard. Bot. Madrid 41: 221. 1984. – Rankender Lerchensporn – Climbing Corydalis, (greek κέρας =horn, due to the horned fruits, the word component *capno*- comes from the Greek καπνος =smoke referring to the similarity with fumewort (*Fumaria*) from Latin *fumus* "smoke". *Clavicula* is latin for tendril or twine).

Homotypic synonyms: *Fumaria claviculata* L. Species Plantarum: 701, 1753, *Corydalis claviculata* (L.) De Candolle, Flore française: 638, 1805, *Capnodes claviculata* (L.) Kuntze, Revisio Generum Plantarum 1: 14, 1891, *Capnoides claviculata* (L.) Druce, Fl. Berkshire: 36, 1897, *Pseudofumaria claviculata* (L.) Büscher & G.H. Loos, Veröff. Bochumer Bot. Ver.: 14 2010

Heterotypic synonyms: no heterotypic synonyms seem to exist.

C. claviculata belongs to the family Fumariaceae DC. (now Papaveraceae), subfamily Fumarioideae (DC.) Endlicher, which sometimes is assigned family level (Stevens 2008). Until 1986 (Lidén 1986) it was assigned to the genus *Corydalis* section *Stylotome* Prantl. Like most species of the section, *C. claviculata* is characterized by fibrous roots, sympodial growth, yellowish flowers, many seeded fruits and a deciduous style which is sharply set off from the ovary. In contrast, the closely related tribe *Fumarieae* (Rchb.) has only one-seeded fruits and an indeciduous style (Fedde 1960). However, it differs from the majority of the *Corydalis* species by developing tendrils and having two cotyledons instead of just one (Fedde 1960).

Lidén (1986) relocated the whole section *Stylotome* to the Mediterranean centered tribe *Fumarieae*. Despite the objections above, the transfer may be justified by the sharing of, e.g., a caducous, chlorophyll-less style, chromosome length, sympodial shoot structure and zygomorphic flowers in

bracteolate racemes. Consequently, the species is assigned to the subtribe *Sarcocapninae* Lidén and the genus *Ceratocapnos* Dur.

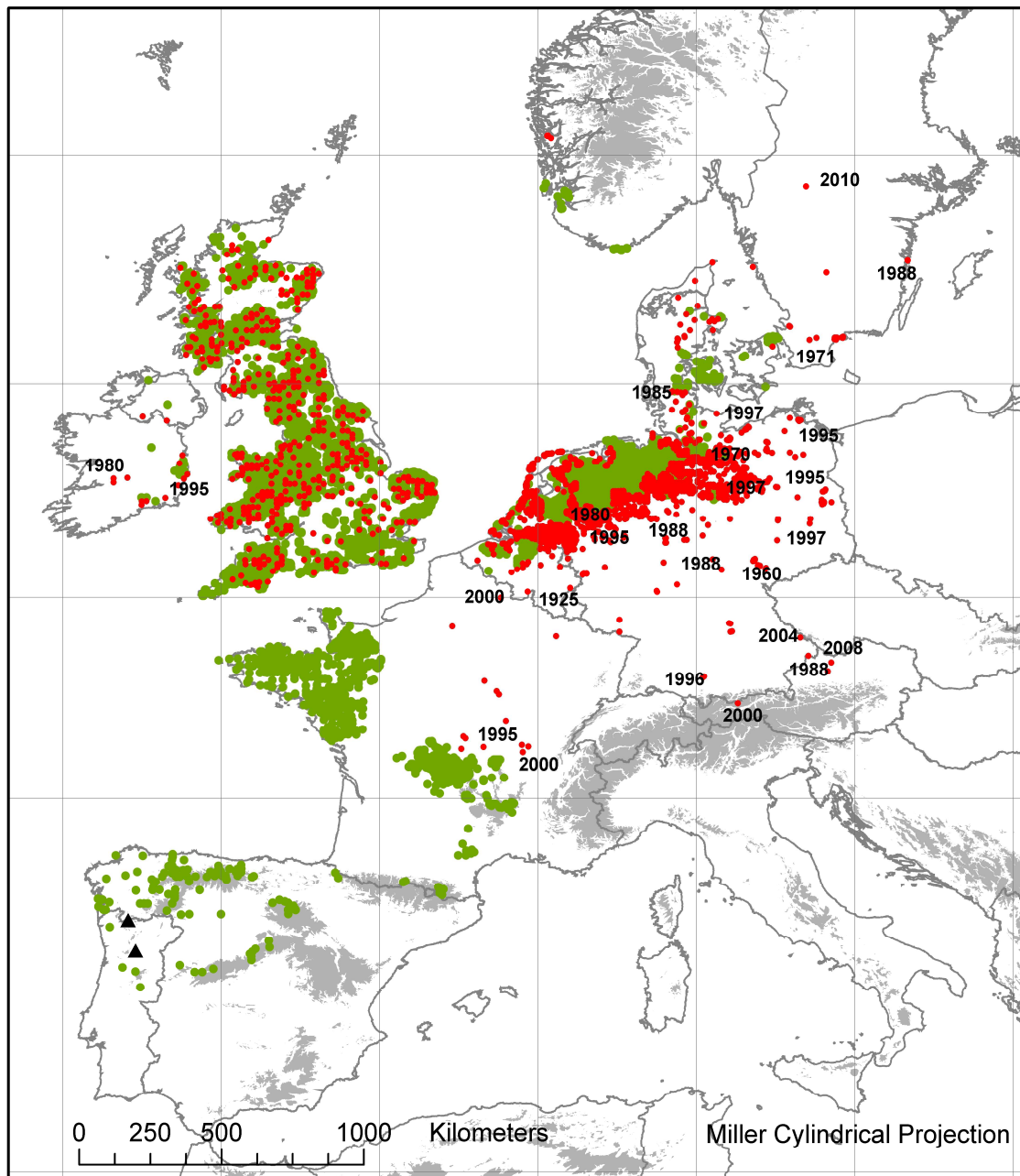


Fig. 1. Geographical distribution of *C. claviculata*. Distribution data were compiled by E. Welk and N. Voss from floristic literature, databases and personal records. Records until 1930 are denoted by green dots; records between 1930-2010 by red dots. Occurrences of the subspecies *C. claviculata* ssp. *picta* are marked with black triangles. For some of the recent records the date of first record is given.

The genus *Ceratocapnos* is recognized by ribbed fruits, filiform hairs and stigma characters (Lidén 1986). Particularly stigma structure is considered taxonomically useful for characterization of tribe, section and genus (e.g. Ryberg 1960, Brückner 1984, Lidén 1986).

The genus comprises only three species, all of them being annual and scandent due to tendrils. While *C. claviculata* is mainly distributed in temperate western Europe (Fig. 1), the other two species, *Ceratocapnos heterocarpa* Dur. and *C. turbinata* (DC.) Lidén are confined to the western and eastern Mediterranean region, respectively. Despite the historical changes of the generic name and uncertainty

of higher taxonomic placement, there was never any taxonomic confusion concerning the identity of the species.

Despite the lack of fibrous clusters in the pericarp, ribs and hairs, the transfer of the species from *Corydalis* to *Ceratocapnos* as a monophyletic group was supported by Fukuhara and Lidén (1995a), who claim that the asymmetrical stigma and the spongy endocarp could be considered an autapomorphy *C. claviculata* shares with the other two species of the genus. However, owing to many similarities between the genera *Pseudofumaria* and *Ceratocapnos* and using a relatively wide definition of genera, Loos (2010) proposed to combine these two genera, retaining the older name *Pseudofumaria* as genus name (see also above, homotypic synonyms).

From the surroundings of Vila Nova de Paiva, province Beira Alta, Portugal, the infraspecific taxon *picta* has been described as a variety by Sampaio (1935). Castroviejo (1998) stated that it could not be recollected again and assigned it to subspecies level (*C. claviculata* subsp. *picta* (Samp.) Lidén). However, there seem to be recent occurrences reported from the Parque Nacional da Peneda-Gerês, province Trás-os-Montes and Alto Douro, Northern Portugal (Vicente 2005), as well as from Parque Natural Baixa Limia Serra Xurés, province Galicia, Spain (Rodríguez and Pereira 2008, Fig. 1). It is very similar to the subsp. *claviculata* but differs in having rose flowers and puberulous capsules due to the presence of small vesicular hairs (Castroviejo 1998). Interestingly, *Ceratocapnos heterocarpa* Dur. and *Ceratocapnos turbinata* (DC.) Lidén also have rose-red petals.

Morphology

Habit:

C. claviculata is a herbaceous, slender, hemirosette, climbing plant species. Due to the weakly developed supporting tissue, the more or less branched, four-angular stem is very weak but can reach up to almost 200 cm length (mean=42.4 cm, Table 1, see also chapter “Life cycle”).

Table 1. Plant traits of *C. claviculata*. Individuals and seeds were collected in the field in 2008. For germination, seeds were exposed for two months to a temperature of 4°C in Petri dishes in a climate chamber. Means and standard errors (\pm s.e.) for the regions north-western Spain (NWE 6 populations), The Netherlands (NL 17 populations), north-western Germany (NWG 19 populations), southern Sweden (S 20 populations), north-eastern Germany (NEG 18 populations) and for the entire range. ANOVA results with F statistic (*F*), degrees of freedom (*df*), p value (*p*) and sample size (*n*) are shown for each measured variable. Superscripted letters indicate significant groups (HSD test). Regions are arranged according to increasing continentality.

	NWE		NL		NWG		S		NEG		Range					
	Mean	\pm s.e.	Mean	\pm s.e.	Mean	\pm s.e.	Mean	\pm s.e.	Mean	\pm s.e.	Mean	\pm s.e.	<i>F</i>	<i>df</i>	<i>p</i>	<i>n</i>
Dry mass, above ground (g)	-	-	0.91 ^b	0.29	1.87 ^b	0.46	0.77 ^b	0.15	0.2 ^a	0.03	0.95	0.14	28.02	3	<0.001	378
Shoot length (cm)	-	-	50.1 ^c	2.72	43.9 ^b	3.34	44.7 ^{ab}	2.89	29.7 ^a	1.91	42.4	1.45	3.420	3	0.022	377
SLA (m ² /kg)	-	-	50.88 ^{ab}	5.03	57.96 ^{ab}	3.53	44.15 ^b	3.76	63.99 ^a	7.07	53.3	2.57	0.193	3	0.048	74
Inflorescences/Individual	-	-	28.8 ^c	7.0	28.7 ^b	6.3	16.5 ^b	2.4	8.6 ^a	1.0	20.8	2.5	4.910	3	0.002	378
Flowers/Inflorescence	8.3 ^c	0.2	7.0 ^b	0.1	6.2 ^a	0.1	7.9 ^c	0.1	5.6 ^a	0.1	6.9	0.1	55.45	4	<0.001	1383
Seeds/Capsule	2.32 ^c	0.07	2.02 ^b	0.03	2.00 ^b	0.04	2.10 ^b	0.04	1.90 ^a	0.04	2.00	0.02	9.840	4	<0.001	1090
Mass 1000 seeds (g)	1.114 ^c	0.024	1.124 ^a	0.012	1.238 ^a	0.014	1.329 ^b	0.009	1.321 ^b	0.009	1.263	0.007	18.96	4	<0.001	198
Germination (%)	21.2 ^a	3.8	53.8 ^b	2.8	71.2 ^c	2.2	55.2 ^b	4.8	46.0 ^b	5.9	54.3	2.3	11.92	4	<0.001	82
Ovules/Flower	2.1	0.2	2.3	0.1	2.4	0.1	2.1	0.1	2.3	0.1	2.2	0.1	1.312	4	0.268	162
Pollen/Flower	412.9 ^a	29.2	151.3 ^a	20.2	509.1 ^a	48.3	512.3 ^a	15.0	413.6 ^a	46.5	488.9	13.0	2.853	4	0.026	162
PO-Ratio	232.7	37.0	238.7	14.2	226.7	26.4	259.3	13.1	195.4	25.3	236.2	8.6	1.628	4	0.170	162

Shoot system:

The shoot system of *C. claviculata* is characterized by sympodial growth (Buchenau 1861, Buttler 1986, Fig. 2). The primary shoot bears five to ten leaves before the apical meristem is terminated by an inflorescence, which is situated in a lateral position in 180° opposition to a leaf. Long before flowering, the uppermost axillary bud continues the mother shoot in direction of the main axis by pushing aside the last raceme; it also terminates after two nodes with an inflorescence. This monochasial branching pattern is repeated for several shoots and eventually finishes when environmental conditions become unfavorable.

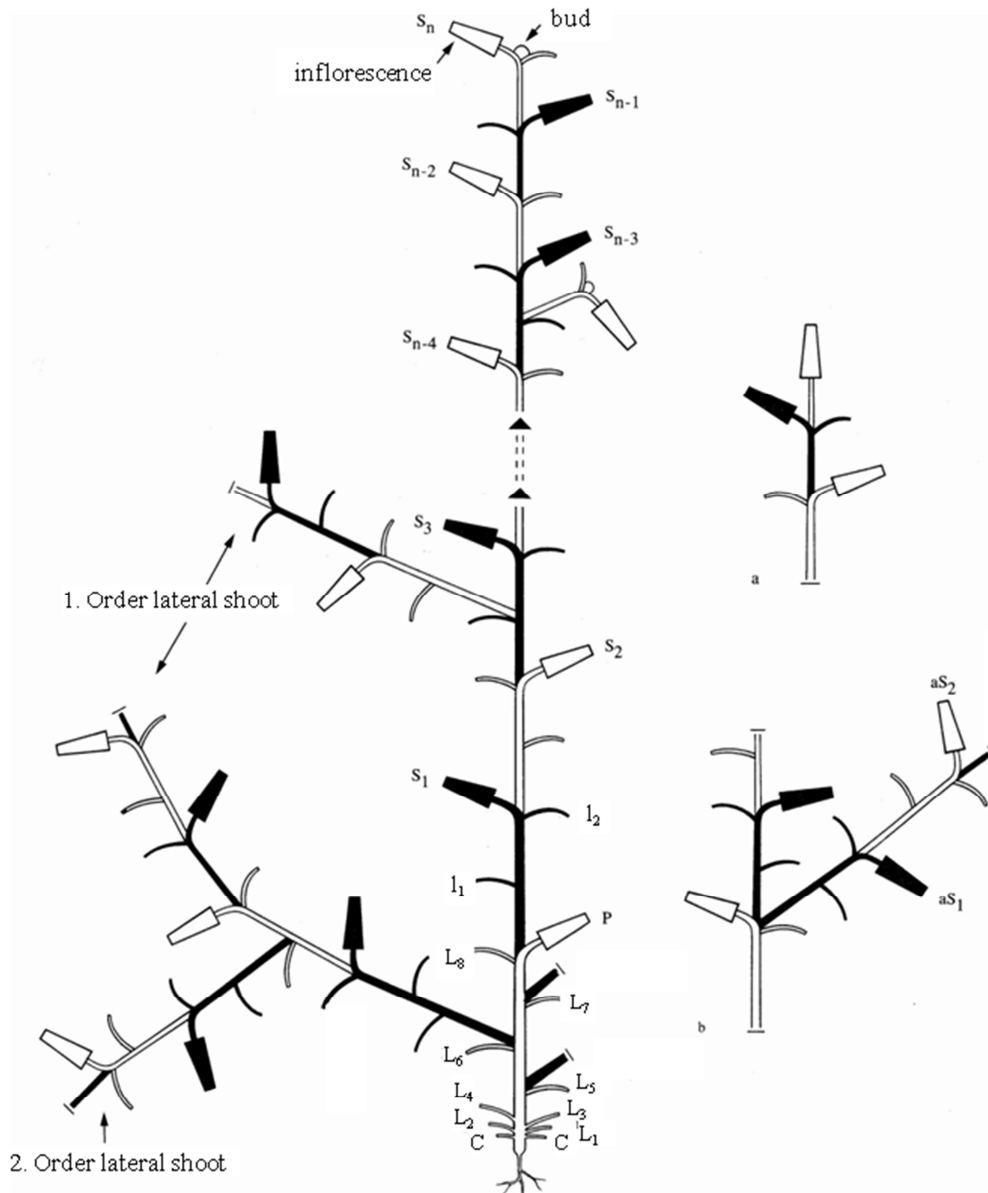


Fig. 2. Diagram of the sympodial shoot system (Buttler 1986). For easier identification, consecutive shoots inclusive of their leaves alternate in black and white. The leading shoot consists of the consecutive primary shoot (=P) and the lateral shoots (=S₁-S_n) forming a shoot sequence: P-S₁-S₂-...-S_{n-1}-S_n. The structure of the first and second order lateral shoots is equal to that of the leading shoot. Leaves of the primary shoot are labeled L₁-L₈, those of the first lateral shoot are labeled l₁-l₂. C=Cotyledons, trapeze=inflorescence, circle=bud. Occasionally there are exceptions to this structure: a) a sequence may directly end with a bud, b) additional buds occur, which may develop into accessory lateral shoots aS₁, aS₂, etc. Figures reproduced with kind permission from the journal "Natur und Museum", modified.

Lower buds that have not taken part in the formation of shoots, i.e. the bud in the first node of the side shoot and in the primary axis in the buds occurring in each of the axils may later grow out as lateral shoots (Ryberg 1960). In *C. claviculata*, buds were found even in the axils of the cotyledons, but only in one case in the greenhouse under optimum conditions those buds could be observed forming shoots (Buttler 1986).

Roots:

The species forms only a sparsely branched shallow root system which is rooting in the humus layer under natural conditions (Lethmate *et al.* 2002, Voss pers. observation). As an annual plant, *C. claviculata* develops no belowground storage organs. The very fibrous primary roots show low cambial growth activity, which soon ceases completely (Ryberg 1960).

Leaves:

The leaves are spirally organized along the stem and rarely pseudo-opposite. They are long petiolate and once or twice pinnate with alternate long leaflets composed of a few entire, elliptic-obovate, tapering segments. These leaflets have three nerves. The number of leaflets increases with length of the shoot. At a stage when the plant comprises two to five leaves, owing to very short internodes, these leaves still grow rosette-like (hemirosette) (Fig. 3). Afterwards, elongation of the stem increases and the internodes can reach up to 5 cm length. The laminae of the segments become smaller in the upper leaves. At the end of the rachis the one to two outer pinnate pairs of leaflets and the final pinnae, petioles and petiolules may strongly elongate and transform into branched tendrils (Fig 3). Often the transformed lamina of the leaflets is still visible as small distal hooks (Goebel 1928, Ryberg 1955, Buttler 1986).

Juvenile *C. claviculata* exhibits only ordinary, unmodified leaves. However, in adult plants any intermediate stage between tendrils and usual leaves can be found. Thus, in the upper region of the plant most of leaves end as tendrils while basal leaves often lack tendrils completely (Ryberg 1960).

The development of tendrils begins in the distal parts of the juvenile rachis, which becomes thinner and longer. The tendrils possess a mechanical sensitivity. If a tendril comes into contact with an object, which serves as mechanical stimulus, it forms a spiral coil in such a direction as to envelope the object, coils around and thus becomes anchored (Darwin 1867, Jaffe and Galston 1968).

The species cannot grow upright by itself unless it attaches to elevated supports during elongation. However, if nothing appropriate can be found, no free coiling occurs and the tip of the tendril bends downwards and inwards and loses its haptotrophic sensibility and power of movement. Thus, only juvenile tissue is able to elongate and coil. The sensitivity decreases stepwise from the tip towards the base of the lamina. The internodes are not sensitive (Darwin 1867).

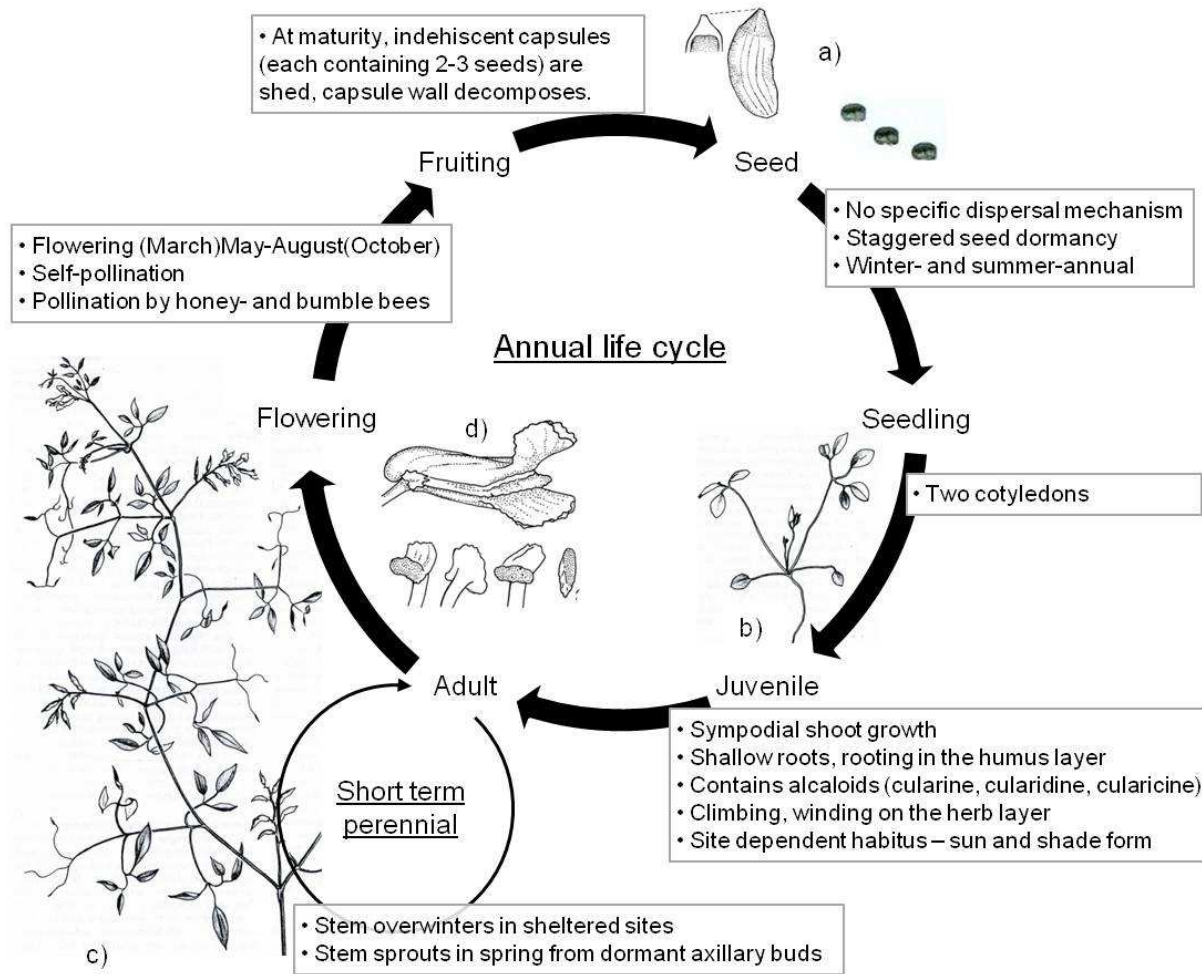


Fig. 3. Life cycle of *C. claviculata*. a) Mature fruit with horned beak and seeds (Lidén 1993). b) Approximately 6 week old juvenile plant with two cotyledons (Buttler 1986). c) Flowering adult plant with tendrils (Buttler 1986). d) Flower and stigma from different perspectives (Buttler 1986, Lidén 1993). Figures reproduced with kind permission from the journal “Natur und Museum” and the project “Flora Iberica”.

Inflorescences:

In *C. claviculata* the flowers are arranged in simple, bracteous, open (polytelic) racemes. The main shoot is terminated by a polytelic raceme and the following partial inflorescences are organized as a monochasial thyrsoidal synflorescence that sometimes is supported by basal paracladia (lateral shoot systems that repeats the structure of the main synflorescence). According to Ryberg (1960) the number of flowers of a single raceme can range between one and thirteen but lies mostly between 6 and 10. We observed inflorescences ranging from a minimum of 1 to a maximum of 20 flowers but 50% of the samples had 5-8 flowers (mean=6.9±0.1 s.e., n=1383, Table 1, see also chapter “Life cycle”).

The racemes are long pedunculate, often with small, tender hairs at the base of the peduncle. The cream-coloured flowers are 5-6 mm long (Lidén 1986, Ryberg 1955, Fig. 3). Schultze-Motel (1986) states that flowers might reach a maximum length of 10 mm but we have never observed flowers of that size. As in *Corydalis* the racemes are bracteolate. At the base of the very short pedicels of each flower there is a small, about 1 mm long, lanceolate bract which is very similar to the scale-like, lapsed sepals (Lidén 1986).

Flower:

C. claviculata has transverse-zygomorphic flowers. The flower consists of four petals in two whorls. The inner, oblong petals are coherent at the tip, jointed about the middle and have well developed median wings. They are dark purple spotted internally, which is not visible from the outside. Internal mottling is found in most taxa in Papaveraceae, but so far, a function is not known. The inner petals are enclosing the androecium and the gynoecium. The outer petals are winged at the apex. The outer, upper petal carries a saccate, downwards curved spur (Ryberg 1955). The spur seems to be on the lower side but in fact originally it was situated sideways and changed its position during development due to torsion of the pedicel by 90° (Fedde 1960).

Gynoecium:

In Papaveraceae exist homo- (fruit opens sutural) and heterocarpellate (fruit opens bivalvate) gynoecia. The gynoecia in Papaveraceae are heterocarpellate since the two carpellae are morphologically not identical. *C. claviculata* has a free superior ovary. It contains 1–4 ovules which are all developing into seeds. The fruit is a capsule with parietal-marginal placentation (Brückner 1984). The style, which is translucent and chlorophyll-free, is sharply set off from the ovary and is separated from it by a distinct incision. A light touch suffices at the end of the flowering season to separate it from the ovary (Ryberg 1960). The stigma consists of a large membranous, oblique dentate crest and an oblong, lateral fleshy structure presumably equivalent to papilla tissue. Except of *Ceratocapnos heterocarpa* no other species within the Papaveraceae has a similar stigma (Ryberg 1960, Fig. 3).

Androecium:

The androecium consists of six anthers carrying eight thecae. There are two stamen bundles, each with one central dithecal and two lateral monothecal anthers. The anthers are rounded-elliptic, closely adhering to the stigma on which the pollen is deposited. So, self fertilization may occur frequently and is supported by the pollen/ovule ratio of the species (see chapter “Reproduction”). However, Hart (1874) observed that at the time of maturity the stigma projects slightly so that it would be first touched by the proboscis of an insect. Furthermore, he suggests that it may be slightly protogynous. The stamen and the petals are connate at the base. In the subtribe *Sarcocapninae* the upper stamen is stouter than the lower and filaments are hyaline. Almost all species of the subtribe *Sarcocapninae* are characterized by panto-colpate pollen with linear-elliptic, often faint, colpi (Lidén 1986). The generalized flower morphology is described by the floral formula $K_2 C_2 + 2 A (1/2+1+1/2) + (1/2+1+1/2) G_2$ (Bresinsky *et al.* 2008). At the base of the stamen there is nectariferous tissue. In all *Fumariae* these nectaries are prolonged and may reach far into the spur. The nectaries are supplied by the central vascular bundle and are partly fused with the spur of the outer petal. Even autogamous species produce nectar, except in depauperate or cleistogamous flowers (Lidén 1986, Lidén 1993).

Seeds:

The seeds are dorsal-apical laterally flattened, smooth and shiny black. With a length of 1.5-2 mm they are relatively large in relation to the size of flowers and fruits (Fukuhara 1999). The seeds are anacampylotropic due to a curved micropyle-hilum-chalaza axis.

The species has a small and flat aril which joins broadly at the hilar region. It is tongue-like, white, fleshy, composed of enlarged cells and reaches to the center of the seed (Fukuhara 1999). However, Cappers *et al.* (2006) does not mention an aril for *C. claviculata* and Ryberg (1955) mentions only a “very inconspicuous” ariloid. Neither in the closely related subtribe *Sarcocapninae* (except genus

Pseudofumaria) nor in the other two taxa of the genus *Ceratocapnos* an aril has been described (Lidén 1986).

Seed coat anatomy:

In general seed coat anatomy is very informative for systematics at the tribal, generic and species levels and thus has been described in detail for Fumarioideae (Brückner 1985, Fukuhara and Lidén 1995b, Fukuhara 1999). *Ceratocapnos* belongs, together with some Corydaleae genera and two other Fumarieae genera (*Cysticapnos* and *Pseudofumaria*), to the same seed coat type. However, taxa belonging to the same seed coat type are not monophyletic, thus the similar anatomy might be a result of parallel evolution (Fukuhara and Lidén 1995b). The cells of the exotesta are wholly filled with dark deposits serving in most Fumarioideae as protective tissue.

Embryology:

At the time of dispersal the seed consists mainly of endosperm while the embryo is still very small and little differentiated. It matures during the following weeks (Buchenau 1861). In Fumarioideae seeds have a multi-layered nucellus (crassinucellate) which contains the embryo sac (Fukuhara and Lidén 1995b). The embryo sac is monosporic (Lidén 1993). In *Corydalis* and *Pseudofumaria* the embryo consists of only two cells at the time of seed shedding and its maturation requires low temperatures (Lidén 1993). Since also in *C. claviculata* cold stratification increased germination to a large extent (see chapter “Germination”), immaturity of the embryo may represent morphological dormancy.

Fruit:

Fruit characters have often been used for subdivision in Fumarioideae (e.g. Fedde 1960, Lidén 1986, Brückner 1992, Fukuhara and Lidén 1995a). *C. claviculata* develops silicular, glabrous, approximately 1 cm long capsules, which are narrowing towards the apex and thus forming a beak (Fedde 1960). This sterile beak is part of the ovary and not of the style (Ryberg 1960). In respect to the pericarp, in Fumarioideae four squarely arranged vascular bundles enter the fruit base; they include two valve-central bundles and two replum bundles. In *Sarcocapnos* and *Ceratocapnos heterocarpa*, clusters of fibres along the valves form longitudinal ribs on the fruit, which are missing in *C. claviculata*.

The endocarp of the fruits envelopes the ripe seeds and sometimes separates from the outer part. In the genus *Ceratocapnos* the endocarp and the innermost layer of the mesocarp are detached together. In *C. claviculata* this applies only for the apical part of the fruits (Fukuhara and Lidén 1995a). In most taxa, the cells of the endocarp are isodiametric and have strongly undulated boundaries. In *Ceratocapnos* this is more pronounced than in other genera, so that the convex parts of the outline protrude like branches and the cells are loosely arranged making the endocarp rather spongy (Fukuhara and Lidén 1995a). The exocarp is thin-walled. In *C. claviculata* also the mesocarp is mainly composed of thin-walled parenchymatic cells. Vascular bundles are distributed in the middle of the parenchyma or near the endocarp. In contrast to other species of *Fumarieae*, *C. claviculata* lacks longitudinal fibers along the vascular bundles and in the mesocarp (not either in *Pseudofumaria*) and clusters of sclerenchyma. However, it has latitudinal fibres that accompany the bundles at the base of the fruit appendage, which explains the indehiscence of the fruits that also occurs in other taxa of *Sarcocapnineae* (Fukuhara and Lidén 1995a). (For the number of seeds see chapter “Life cycle”).

Distribution and habitat requirements

Geographical distribution

C. claviculata is an eu-oceanic species native to western Europe. The geographical distribution ranges from northern Portugal in the south-west to northern Scotland in the north-west, southern Norway in the north and from easternmost Denmark in the north-east to southern France in the south-east (Fig. 1). The species is reported to be native from the following European countries: Portugal, Spain, France, Ireland, United Kingdom, Belgium, Netherlands, Germany, Denmark, and Norway. Non-native occurrences are reported from Sweden (Oredsson 2005) and, quite recently, from Austria (Kleesadl 2009). The highest densities of *C. claviculata* populations are reported from Great Britain, North-western France, Belgium, The Netherlands, and north-western Germany. Thus, the European Atlantic floristic province of Takhtajan (1986) represents the current range centre of the species, and the general distribution can be described as submeridional to temperate within the continentality regions 1-2 in Europe. The respective range formula after Jäger and Werner (2005) reads sm/mo-temp c1-2 EUR.

In the Temperate floristic zone, the species occurs from the planar to the colline altitudinal zone, yet is predominantly confined to lowlands (Fig. 4, 80% of occurrences between ca. 0 m and 300 m asl.). In the submeridional floristic zone, extending northwards to approximately 46° N in western Europe, a clear shift toward montane habitats is visible (Figs. 1 and 4). This sharp altitudinal shift is enforced by the geographical disjunction between the lowland occurrences in north-western France and the occurrences in the Massif Central. Additionally, there is a higher amount of altitudinal variation visible in this range part, since, e.g. in Spain, the species grows in the lowlands of coastal, air-humid Galicia from 80-120 m (Castroviejo 1975) but also in the Picos de Europa from 1200-1500 m (Rivas-Martínez *et al.* 1984) and in the Sierra de Guadarrama at altitudes of up to 1750 m (Fernández-González 1991) probably because in the interior territories air-humid climates are confined to higher altitudes.

Several other plant species share a similar general distribution pattern and are therefore classified as a range type or floristic element. Meusel *et al.* (1965) assign *C. claviculata* to the *Ulex* range type. This type contains species of atlantic heathlands, acidic mires and woodlands that are completely or mostly confined to the Atlantic floristic province. The name giving species *Ulex europaeus* is similar to *C. claviculata* amongst the Oceanic Temperate species as it has also spread from its native range eastwards into Europe, and now has a Suboceanic Temperate distribution. Further species with similar distribution are *Wahlenbergia hederacea* (L.) Rchb., *Lobelia urens* L., *Cirsium anglicum* D.C., *Sedum anglicum* Huds., *Scilla verna* L., or *Hypericum helodes* L. Thorough study of this plant distribution range type has been provided by Dupont (1962) and Roisin (1969). The climatic features were summarized by Gimingham (1972) and can be best described as cool temperate conditions with high rainfall and low annual temperature oscillation. Species of this range type seem to avoid both, areas with low air humidity in the vegetation period and very low winter temperatures and thus are generally absent from mediterranean and continental areas. In a recent ecogeographical analysis of the European Atlantic heathlands (Loidi *et al.* 2010) ordinations showed that the main climatic factors determining heathland floristic distribution are thermicity (thermicity index; $[(T+M+m) \times 10]$, *T*: yearly average temperature, *M*: average maximum temperature of the coldest month of the year, *m*: average minimum temperature of the coldest month of the year) and summer drought (evapotranspiration for the 3 summer months).

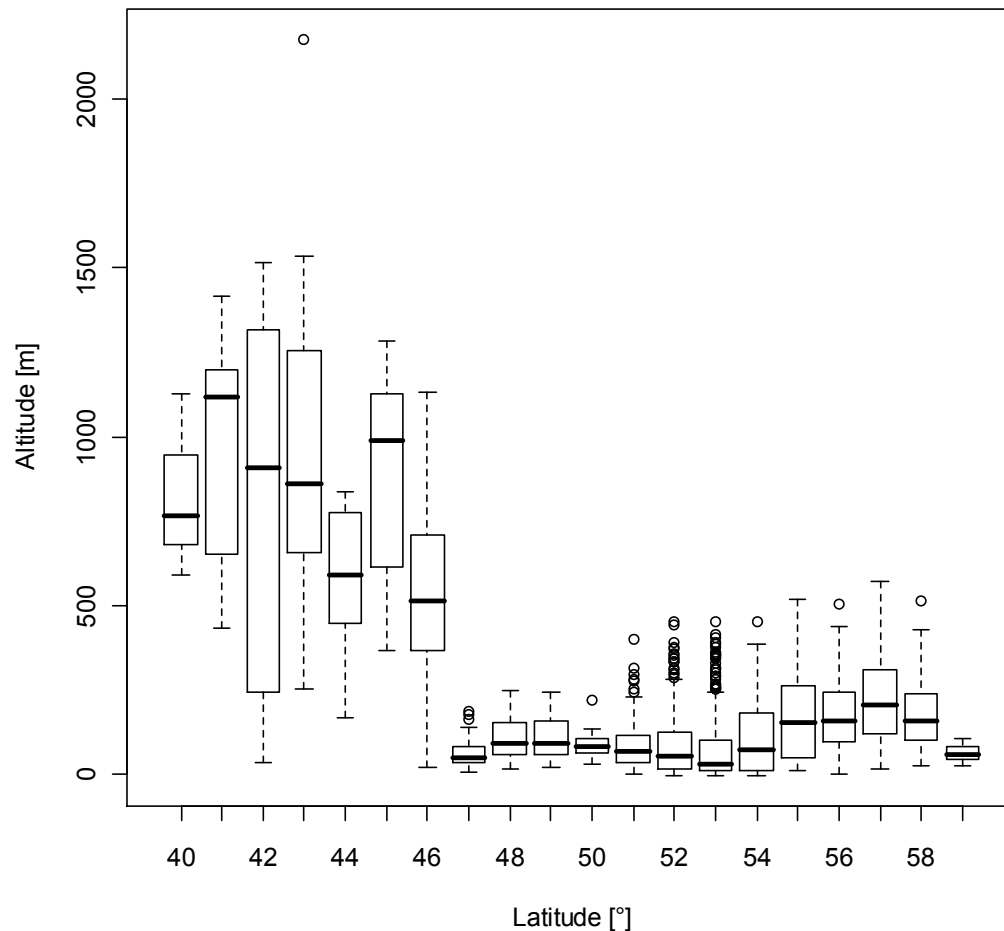


Fig. 4. Box and whisker plots of the altitudinal distribution of *C. claviculata* along latitude classes of one degree. Data are extracted from the compiled distribution data. Indicated are the lower and upper quartiles (box), the median (bold line), as well as the 1.5x interquartil range (whiskers), outliers mostly based on data resolution issues are removed.

Given the supposed frost sensitivity and the relatively high demands on humidity, the question arises where *C. claviculata* might have persisted during the Quaternary glacials, especially the Last Glacial Maximum. Nearly all phylogeographic studies for Europe focused on species that are supposedly far more cold resistant than *C. claviculata*. Mahy *et al.* (1999) report that *Calluna vulgaris* might have survived in two regions during the last glaciation events: south-western Europe and south Britain. For *C. claviculata*, only the supposed south-western Iberian pleistocene refugium for temperate species might be regarded as suitable. The re-colonization of western Europe probably might have originated from refugia considerably south of the species' current distribution range. Migrants from this refugium were the likely source of colonists for populations occupying the previously or meanwhile unsuitable lowland habitats along the north-eastern Atlantic Coasts. Since all the above hypotheses remain highly speculative, phylogeographic investigations using molecular markers are urgently needed for a better understanding of both, the distributional history and future of the species.

Habitat

Regarding light requirements, in the indicator value evaluation of Ellenberg *et al.* (1992) the species obtained a light value of 5 and thus mainly prefers semi-shaded sites. Indeed, most populations occur in semi-shaded open oak-, birch-, and pine-forests, in fringes, hedgerows or ditches along roads. The species also thrives very successfully on sun exposed forest clearings (Buchenau 1861, Tüxen and Jahns 1962, Buttler 1986, Fukarek and Henker 2006).

On the other hand, in the UK *C. claviculata* grows in mixed woodland with a canopy cover of at least 20% (Hill *et al.* 2004). However, in dark beech forests, unfavorable light conditions seem to prevent the colonization by *C. claviculata* and in the course of succession from open to more shady habitats the species usually declines (e.g. Pollmann and Lethmate 2006, chapter 2, but see Benkert *et al.* 1995).

The preferential occurrence in half-shady locations is also in line with the species' water requirements: in central Europe, *C. claviculata* usually occurs on fresh soils of intermediate moisture (Lethmate *et al.* 2002). Also in the south-western part of its range, in northern Spain and south-western France, the species grows in forests and hedges on fresh soils but it may also be found on walls and more or less shady, rocky sites with an inclination of 0-10% (Castroviejo 1998, Dupont 2001). In this kind of habitat, humus accumulating crevices may favor therophytic pioneers like *C. claviculata* but the shallow soil layer may become very dry during summer (Clement and Touffet 1977). The occurrence of the species on sun exposed sites and rocky slopes contrasts with its slender, hygromorphic habit and its poorly developed, little-branched root system, predominantly rooting in the surface layer. However, low water storing capacity of these habitats may be compensated by low evapotranspiration rates due to the atlantic climate with high air humidity through mist or high amounts of precipitation during the time of germination (autumn-spring) and growth (spring-early summer). The annual precipitation e.g. in the atlantic zone of northern Spain is between 900-1800 mm with a mean annual temperature of 5°C in the subalpine and 9.3°C in the montane zone (Rivas-Martinez *et al.* 1984). One of the southernmost occurrences of the species lies in the Sierra de Guadarrama in Spain. Here, *C. claviculata* is found only in a few north-exposed mountainous beech forests. Soils in these habitats have a good water supply due to annual precipitation sums of between 1000 and 1400 mm and an annual mean temperature of only 6–10°C (Fernández-González 1991). In the native range, the lowest long term mean values of annual precipitation of about 540–550 mm are to be found at occurrences in the East-Anglia counties of south-eastern England. The driest regions within the broadened synanthropic range are the sub-oceanic regions Saxony-Anhalt and Saxony (eastern Germany) where the species is found in forests and degenerated bogs (Rathey 1984, Kühn and Gutte 1997), and in south-eastern Sweden (Kalmar county), where long term means of 500 to 530 mm precipitation are calculated (SMHI 2006, DWD 2010).

Obviously, the lower values for precipitation and temperature are quite similar between the native and the recently invaded range. Indeed, when testing monthly mean values for precipitation and temperature and bioclimatic variables (Hijmans *et al.* 2005), Isothermality and MTWQ (mean temperature of the wettest quarter) were the only variables that differed between native and non-native occurrences to a larger degree. Both variables are connected with changes from oceanic to suboceanic-subcontinental climate. Towards the east and away from the Atlantic and North Sea coasts the temperature differences between summer and winter increase, as well as the proportion of summer rain at the annual precipitation sum (Welk, unpubl.).

C. claviculata is mostly growing on humic, acidic and moderately acidic soils of intermediate to high fertility (Ellenberg *et al.* 1992, Hill 2004). The species roots in the organic surface layer, which consists mostly of moder or raw humus, or of hardly decomposed litter formed by needles, and leaves of trees or grasses (Passarge and Hofmann 1968, Decocq 2000, Lethmate *et al.* 2002, Horstmann 2005). The thickness of this layer may range from about 1-30 cm (Voss unpublished data).

In The Netherlands, Germany, Belgium, Sweden and the UK, the subsequent mineral soil often is dominated by a sandy to silty fraction of low fertility. Across the species' range various types of bedrock can be found such as quartz sand, sandstone, granite, gneiss, crystalline or schistous rocks. The soil types developing on these substrates range from acid brown soils to podzols and to pseudo- and stagnogleys (Tüxen and Diemont 1937, Rodwell 1998). The shallow soils on rocky slopes of quartzite and schist in France and Spain are mostly developed as ranker (Provost 1993, Castroviejo

1998, Dupont 2001). Typically all soils mentioned above have a low base status and are acidic with pH values from less than 3.5 to 4.6 (Clement *et al.* 1980, Rodwell 1998, Dengler 2007, Voss unpublished data). In general, the species is absent from limestone areas unless it is overlain by non-calcareous substrate as described by Horstmann (2005) for the occurrence of the species on calcareous silty loam, which was covered by 20–25 cm of raw humus so that the lime did not affect the shallow-rooted herbal layer.

This preference for acidic soils seems to be one of the main reasons for the disjunct distribution of the species in France. Here, the northern range part is sharply limited by Cretaceous and Jurassic parent materials surrounding the Paris basin. A comparison of the distribution pattern with geological maps indicates that *C. claviculata* is confined to islands of geologically acidic parent materials of Cambrian and Pre-Cambrian age (Hercynian domain). A lower frequency of occurrence is also recognizable for the Jurassic region in England. Following from this geological constraints, the Vosges region in easternmost France might potentially provide geologically suitable habitats for the species. Interestingly, acidic soil types of different parent material that occur abundantly in regions like Haute-Normandie, Centre (Loire, Loire-et-Cher, Cher), and Aquitaine (Gironde, Landes), are not populated by the species (Welk, unpubl.).

C. claviculata typically occurs on leached, oligotrophic soils (e.g. Passarge and Hofmann 1968, Rattey 1984). However, during the last 50 years nitrogen availability in soils increased as a consequence of atmospheric deposition (Bobbink *et al.* 2010). Growth and competitive effect of *C. claviculata* seems to be promoted by this process (van der Eerden 1998, Lethmate *et al.* 2002). Additionally, soil disturbances and open patches in the herb layer of forests resulting from logging, tree fall gaps and, to a smaller extend, activity of animals may enhance nutrient availability through accelerated rates of mineralization (Brady and Weill 1999).

C/N ratios of the humus layer found across the range on forest locations were around 30 in the Teutoburger Forest (Lethmate *et al.* 2002), 26 in eastern Brandenburg and 24 in north-western Germany, 24 in central eastern part of the Netherlands and 25 in southern Sweden (Voss unpublished data). On shallow podzolic soils in France in Brittany with up to 20 cm thick organic layer there was a ratio of 21–25 (Clement *et al.* 1980).

Communities

In order to get an impression of the species composition in the different parts of the entire range of the species we assigned 920 vegetation relevés (for details see chapter 2) with *C. claviculata* from across the entire range to five climatic environmental zones (Metzger *et al.* 2005, for the zones see Table 2). These zones differed in respect to minimum and maximum temperature of the months January, April, July and October, precipitation during these months and oceanity (Metzger *et al.* 2005). For each zone the ten most frequent species of the herb/shrub layer and the five most frequent species of the tree layer were identified (Table 2). The most frequent (> 25%) herb/shrub species across the entire range associated with *C. claviculata* were *Deschampsia flexuosa*, *Sorbus aucuparia*, *Quercus robur*, *Rubus fruticosus* agg. plus *corylifolius* agg., *Pteridium aquilinum*, *Frangula alnus*, *Holcus mollis*, *Rubus idaeus*, *Lonicera periclymenum*, *Stellaria holostea*, *Teucrium scorodonia*, *Dryopteris dilatata*, *Agrostis capillaris*, *Dryopteris carthusiana*, and *Vaccinium myrtillus*. The most frequently occurring (> 10%) tree species were *Quercus robur*, *Fagus sylvatica*, *Pinus sylvestris*, *Betula pubescens*, *Betula pendula*, and *Ilex aquifolium*.

Table 2. Frequency (%) of the ten and five most frequent species (bold numbers) co-occurring with *C. claviculata* in the herb/shrub and tree layer (indicated by “T”), respectively, in five climatic environmental zones (Metzger *et al.* 2005) from across the entire range and the means of all groups. In deviation to Metzger *et al.* (2005), the zone Alpine South s.l. here comprises the original zones Alpine South, Mediterranean Mountains and Mediterranean North.

Zone	Continental	Atlantic North	Atlantic Central	Lusitanian	Alpine South s.l.	Mean
Number of relevés	110	259	444	69	38	920
Total species number	203	203	260	173	155	382
<i>Betula pendula</i> (T)	10	11	11	22	0	11
<i>Betula pubescens</i> (T)	15	18	7	4	24	14
<i>Castanea sativa</i> (T)	0	0	2	16	3	4
<i>Fagus sylvatica</i> (T)	7	31	9	28	34	22
<i>Ilex aquifolium</i> (T)	0	1	1	22	29	11
<i>Picea abies</i> (T)	9	12	2	1	0	5
<i>Pinus sylvestris</i> (T)	54	22	24	0	11	22
<i>Quercus robur</i> (T)	8	57	35	46	13	32
<i>Robinia pseudoacacia</i> (T)	13	0	0	1	0	3
<i>Sorbus aucuparia</i> (T)	2	6	3	7	29	9
<i>Agrostis capillaris</i>	34	16	23	43	18	27
<i>Deschampsia flexuosa</i>	87	50	50	62	32	56
<i>Digitalis purpurea</i>	5	5	19	48	24	20
<i>Dryopteris carthusiana</i>	55	46	30	3	3	27
<i>Dryopteris dilatata</i>	21	56	39	4	18	28
<i>Dryopteris filix-mas</i>	6	5	7	33	37	18
<i>Erica arborea</i>	0	0	0	7	47	11
<i>Frangula alnus</i>	36	48	45	23	8	32
<i>Galeopsis tetrahit</i>	19	31	24	38	3	23
<i>Galium saxatile</i>	20	16	12	33	16	20
<i>Holcus mollis</i>	8	22	30	65	37	32
<i>Lonicera periclymenum</i>	14	43	27	32	34	30
<i>Molinia caerulea</i>	17	35	31	9	0	18
<i>Prunus serotina</i>	23	25	35	0	0	17
<i>Pteridium aquilinum</i>	1	20	28	45	76	34
<i>Quercus robur</i>	55	54	50	17	3	36
<i>Rubus fruticosus</i> + <i>corylifolius</i> agg.	44	66	41	1	21	35
<i>Rubus idaeus</i>	59	47	11	35	11	33
<i>Sambucus racemosa</i>	25	2	3	9	0	8
<i>Sorbus aucuparia</i>	72	75	46	28	3	45
<i>Stellaria holostea</i>	7	25	10	38	66	29
<i>Teucrium scorodonia</i>	0	3	25	49	61	28
<i>Vaccinium myrtillus</i>	17	37	17	17	42	26
<i>Viola riviniana</i>	0	2	3	17	34	11

C. claviculata occurs in various forest communities in different parts of its range and is considered as character species of the acidophytic birch-oak forests in the order *Quercetalia roboris* Tx. 1931. In the map of the natural vegetation of Europe (Bohn *et al.* 2002/2003) all communities in which *C. claviculata* occurs belong to the macroclimatic zone “mesophytic deciduous broadleaved forests and mixed coniferous-broadleaved forests”. This formation contains subgroups that differ with respect to species composition, climatic gradients and large scale habitat factors. These groups, in turn, are subdivided into different types with respect to nutrient regime, altitude, moisture regime and geographic location.

C. claviculata is diagnostic species or occurs as frequent species in the subgroups 1. “species poor acidophilous oak and mixed oak forest” (here the species occurs in communities at all altitudes), 2. “mixed oak ash forests” and 3. “species poor oligotrophic to mesotrophic beech and mixed beech forests”.

1. Species poor acidophilous oak and mixed oak forest,

lowland–colline (to submontane) types:

- Atlantic-subatlantic birch-pedunculate oak forests (=F8 in Bohn *et al.* 2002/2003) in Belgium, Denmark, north-western Germany, southern Norway, southern Sweden, The Netherlands and the central UK. As diagnostic species there are e.g. *Quercus robur*, *Betula pendula*, *Deschampsia flexuosa*, *Melampyrum pratensis*, *Hieracium laevigatum*, *Lonicera periclymenum*, *Dryopteris carthusiana*, *Dryopteris dilatata*, *Vaccinium myrtillus*.
- Atlantic-subatlantic hygrophilous birch pedunculate oak forests (F9) in northern France, northern Germany and The Netherlands with diagnostic species such as *Q. robur*, *L. periclymenum*, *Frangula alnus*, *Erica tetralix*, *Molinia caerulea*, *Pteridium aquilinum*, *Aulacomnium palustre*, *Polytrichum commune*.

Colline-submontane types:

- Galician-north Lusitanian hyperoceanic pedunculate oak forests (F14) in north-western Spain and north-western Portugal with e.g. *Q. robur*, *Quercus pyrenaica*, *Ruscus aculeatus*, *Agrostis castellana*, *Daboecia cantabrica*, *Saxifraga spathularis*, *Viola riviniana*, *Blechnum spicant*.

Montane and altimontane types:

- Orocantabrian-north-west Iberian altimontane birch sessile oak forests and birch forests (F25) in northern Portugal and north-western Spain with e.g. *Quercus petraea*, *Betula pubescens* ssp. *celtibérica*, *Erica arborea*, *I. aquifolium*, *V. myrtillus*, *Doronicum carpetanum*, *Luzula sylvatica* ssp. *henriquesii*, *B. spicant*.

2. Mixed oak ash forests, colline-submontane types:

- Cantabrian-Euscaldian mixed oak-ash forests (F33) in northern Spain with e.g. *Fraxinus excelsior*, *Q. robur*, *Crataegus monogyna*, *C. avellana*, *Brachipodium sylvaticum*, *Arum italicum*, *Pulmonaria longifolia*, *Stellaria holostea*.

3. Species poor oligotrophic to mesotrophic beech and mixed beech forests,

lowland (-colline) types:

- Norman-Belgian (sessile oak-) beech forests (F76) in northern France, Belgium, The Netherlands with *F. sylvatica*, *Q. petraea*, *I. aquifolium*, *L. sylvatica*, *Digitalis purpurea*, *Rhytidiadelphus loreus*.

colline-submontane types:

- Atlantic-subatlantic *Luzula sylvatica*-(sessile oak-) beech forests (F82) of the French Massif Central with *F. sylvatica*, *I. aquifolium*, *Luzula forsteri*, *Euphorbia hyberna*, *Doronicum spec.*, less frequent *Q. petraea* (Bohn *et al.* 2002/2003).

As vegetation types in this system are relatively coarsely defined for western and central Europe which are the main regions of distribution of *C. claviculata*, we present these here in more detail:

In acidophytic birch-oak forests *C. claviculata* has its main occurrence in the associations *Deschampsio flexuosae-Quercetum roboris* Passarge 1966 (=F8 in Bohn *et al.* 2003) and *Betulo pendulae-Quercetum roboris* Tx. 1930 nomen inversum propos (=F8 und F9 in Bohn *et al.* 2003). In contrast to the latter, the *Deschampsio flexuosae-Quercetum roboris* Passarge 1966 is characterized by relatively dry, nutrient poor and little developed soils and lower species number. The *Betulo pendulae-Quercetum roboris* Tx. 1930 nomen inversum propos features boreal species such as *V. myrtillus* and partly *V. vitis-idaea*, *Maianthemum bifolium* and *Trientalis europaea* (Härdtle *et al.* 1997). In the UK, *C. claviculata* occurs in similar, atlantically distributed associations, namely the acidophytic communities of the *Quercus robur-Pteridium aquilinum-Rubus fruticosus* woodland on oligo- to mesotrophic brown earth of low base status or ranker, and the *Quercus* spp.-*Betula* spp.-*Deschampsia flexuosa* woodland on oligotrophic podzols and stagnogley-podzols, often under slightly colder and moister climate (Rodwell 1998, =F8 in Bohn *et al.* 2003). In France and Belgium, the species occurs in acidophytic, atlantic beech forests of the alliance *Ilici-Fagion* Braun-Blanquet 1966 (=F76 and F82 in Bohn *et al.* 2003). However, most of these stands are also rather *Betulo-Quercetum* communities (Härdtle *et al.* 1997, Heinken, pers. comm.).

In the course of changes of the forest management during the past century, in north-western and central Europe many original or near natural forests have been replaced by conifer plantations in the class *Vaccinio-Piceetea* Br.-Bl. in Br.-Bl. *et al.* 1939 (with spruce, douglas fir, larch, pine) or, but less frequently, plantations of *Robinia pseudoacacia*. Both forest types form substitute communities but exhibit partly a similar understorey composition (e.g. Rodwell 1998, Decocq 2000, Berg *et al.* 2001, but see Heinken 1995). These conifer plantations occur on peat soils of disturbed bogs (Meyer and Voigtländer 1996), on heathlands and inland dunes on sandy soils (Heinken 1995). For the latter, pine forests of the type *Dicrano-Pinion* are characteristic and play a major role for the occurrence of *C. claviculata*. The species is characteristic for *Deschampsia flexuosa-Pinus sylvestris*-communities of relatively nitrogen and humus rich locations (Heinken 1995). Within these it characterizes in Germany the atlantic(-boreal) *Galium saxatile*-vicariant (Heinken 2008).

Although birch-oak forests are the most characteristic near-natural community types across the entire range, *C. claviculata* occurs often in higher abundance than in ecotonal communities or under disturbed conditions (see chapter “Abundance”). Within the acidophytic forest clearing communities, stands with *C. claviculata* comprise the association *Corydalis claviculatae-Epilobietum angustifolii* Hülbusch & Tx. 1968 (Berg *et al.* 2001, 2004, Dengler *et al.* 2007); closely related communities are found in the UK or in France (Rodwell 1998, Decocq 2000). Another, permanent derivative of clearing and old coppice stands described for the UK is represented by the *Pteridium-Rubus* underscrub where *P. aquilinum* can form the virtually completely closed canopy of the vegetation (Rodwell 1998). Further ecotonal communities outside forests are along forest edges, hedges, roads, ditches and tracks. These stands may be assigned to different syntaxa within the *Origanetalia vulgaris* Th. Müller 1961, *Convolvulion sepium* Tx. 1947 and *Alliarion* Oberd. (1957) 1962 (Castroviejo 1975, Jäger and Werner 2005, BfN 2010). Finally, the species occurs in dwarf-shrub heaths and sometimes even in pioneering, xerophilous communities on schist outcrops but under high air-humidity. In Brittany, these occurrences were attributed to the *Erico cinereae-Vaccinietum myrtilli* and the *Festuco-Sedetum anglici* (Clement and Touffet 1977, Clement *et al.* 1980).

Response to abiotic factors

Due to its slender, hygromorphic habit the species is confined to either damp, but well aerated soils or sites of high relative humidity (see chapter “Habitat”). Especially seedlings respond very sensitive to drought. In a greenhouse experiment, germination was significantly higher in pots that were kept constantly moist than in those that were left to fall intermittently dry ($47.2 \pm 1.9\%$ vs. $23.2 \pm 2.7\%$, mean \pm s.e.; t-test=7.189, df=18, $p < 0.001$) (Voss unpublished data). These results are consistent with field

observations: Germination success was significantly higher in populations in the native, oceanic range than in plots in the more suboceanic, invaded range which might be due to lower precipitation in the invaded range sites resulting in dry conditions during spring (Voss unpublished data). However, adult individuals are able to tolerate drought despite their shallow roots as we observed vital, fruiting individuals (Fig. 5) on clearings without tree cover (average cover of *C. claviculata*: 8 vs. 4% in forests with a mean tree cover of 45%) and in common garden pot experiments (Voss unpublished data).



Fig. 5. *C. claviculata* under different light conditions. a) Habitus in a shaded forest site, b) Habitus in a sun-exposed forest clearing.

Under field conditions, we found significant differences in specific leaf area (SLA, m^2kg^{-1}) between leaves of plants in shady, half-shady and sun-exposed sites in a population in southern Sweden (Fig. 6). North-eastern German localities were darker than the other sites which was reflected by a mean SLA of $63.99 \text{ m}^2\text{kg}^{-1}$. In contrast, in Swedish plants there was a mean SLA of $44 \text{ m}^2\text{kg}^{-1}$. This is equivalent to sunny and half-shaded sites and in fact, many of the Swedish populations were situated in forest clearings. Furthermore, individuals exposed to different light conditions were characterized by an overall different habit (Fig. 5). The development of thicker, more succulent summer and thinner winter leaves is also found in the closely related genus *Sarcocapnos* (Lidén 1986).

In a controlled common garden experiment, we tested the effect of sun exposure on the response traits germination, SLA, number of inflorescences, fruits and seeds, length, biomass, and seed mass through comparison of plants in sun-exposed (corresponding to situations in treeless open areas) and shaded pots (shading corresponding to situations in open forests). In order to take into account effects of geographic variability, seeds used for this experiment were obtained from plants of Spanish, Dutch, north-western German, north-eastern German and Swedish origin. In order to avoid maternal effects parental plants were grown in the greenhouse (for details see chapter “Germination”). The soil (Fruhstorfer Erde Typ P, Hawita-Gruppe, Vechta) that we used in the experiment contained 160 mg/l nitrogen. Germination was significantly higher in shaded pots, which probably was due to a higher probability of desiccation in sun-exposed pots (Table 3).

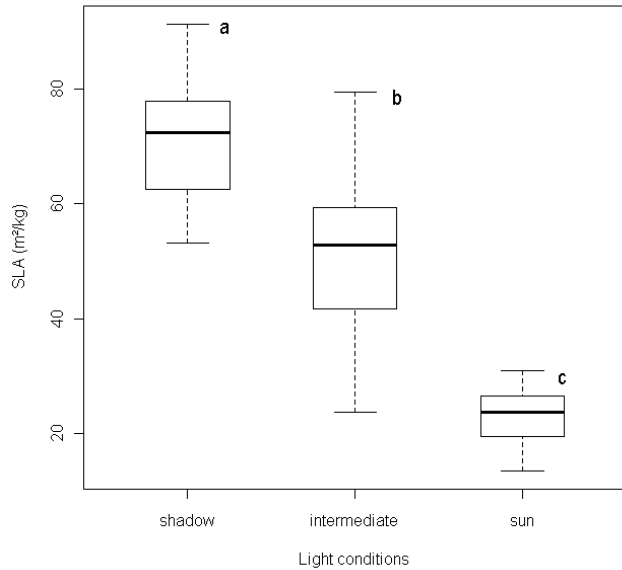


Fig. 6. Impact of light on specific leaf area (SLA, m^2kg^{-1}) of *C. claviculata*, measured in plants from shaded (whole day shaded), half-shaded (edge of the forest) and sun-exposed sites (clearing) in a population in southern Sweden. Shown are the median, the lower and the upper quartile and minimum and maximum values. Letters a-c indicate significant groups (HSD test). ANOVA: $F_{2,73}=50.216$; $p<0.001$.

Under sun-exposed conditions SLA was significantly smaller than for shaded plants. Furthermore, sun-exposed individuals flowered earlier than shaded plants. By mid-May, 61 % of the sun exposed individuals were flowering but only 41 % of the shaded individuals. Two weeks later all individuals were abloom.

With respect to shoot length, shaded plants were significantly longer than sun exposed plants (Table 3). The number of seeds per capsule was slightly but significantly higher in plants of sun exposed pots and also the number of capsules was higher. In turn, seed mass was higher in shaded plants. However, no differences between the two treatments were found for number of inflorescences/individual and for above ground biomass.

In the same experiment, we studied the effect of the application of either 5 or 50 kg N per ha and year. There were no differences between fertilizer levels with respect to germination and shoot length. However, there was a positive

response to fertilization of the number inflorescences per plant, biomass, and the number of seeds per capsule (Table 3).

(For information on soil requirements see chapter “Habitat”, for response to low temperature see chapters “Phenology” and “Germination”).

Table 3. Effects of the factors light (sun, corresponding to situations in treeless open areas, vs. shade, corresponding to situations in open forests) and nitrogen (application of 5 vs. 50 kg N $\text{ha}^{-1} \text{year}^{-1}$) on different fitness parameter of *C. claviculata*. Shown are mean, standard error ($\pm\text{s.e.}$), F statistic (F), p value (p) and sample size (n).

Response variables	Sun		Shade		F	p	n
	mean	$\pm\text{s.e.}$	mean	$\pm\text{s.e.}$			
Germination (%)	28	23	12	11	103.7	<0.001	111
Shoot length (cm)	55.9	1.2	90.7	1.5	323.2	<0.001	95
SLA (m^2kg^{-1})	35.3	1.2	75.3	2.3	333.8	<0.001	98
Capsules/inflorescence	9.7	0.3	8.8	0.2	11.91	<0.001	93
Seeds/capsule	2.19	0.06	2.00	0.04	6.122	0.015	99
1000 seeds (g)	1.088	0.022	1.004	0.018	19.12	<0.001	87

Response variables	5kg N $\text{ha}^{-1} \text{year}^{-1}$		50kg N $\text{ha}^{-1} \text{year}^{-1}$		F	p	n
	mean	$\pm\text{s.e.}$	mean	$\pm\text{s.e.}$			
Biomass (g)	3.7	0.2	2.7	0.2	12.82	<0.001	92
Inflorescences	73.4	3.8	55.3	3.4	10.77	0.001	98
Seeds/capsule	2.2	0.1	2.0	0.1	3.573	0.062	99

Abundance

In forests *C. claviculata* occurs with abundances ranging between 1 and 4% (references denoted by their IDs from the Global Index of Vegetation-Plot Databases (GIVD), Dengler *et al.* 2011; Heinken 2012, ID EU-00-008; Rodwell 2012, ID EU-GB-001 and Peppler-Lisbach 2012, ID EU-DE-010).

However, in ecotonal communities, such as clearings, the species occurs with an average abundance of about 35% (Dengler *et al.* 2007). Especially on young clearfellings the entire area inclusive tree trunks may be covered by vital, intensely blooming, huge individuals, so that in places *C. claviculata* may be the most dominant plant species (Dengler *et al.* 2007, Voss personal observation).

Life cycle and biology

Life cycle

C. claviculata is predominantly a summer- but also winter-annual semirosette therophyte (Buchenau 1861, Voss unpublished data). Due to this potential overwintering it is described as therophytic and partly as hemicryptophytic species (Moffat 1923, Griffioen 1961, Jäger and Werner 2005). Seedlings of *C. claviculata* may emerge directly after dissemination in autumn (September) (Fig. 3). Under moist conditions in the greenhouse, we observed seeds that germinated only two weeks after dissemination. However, most of the seeds will not germinate before spring owing to their requirement for cold stratification to break seed dormancy (see also chapter “Germination”). The species propagates by seeds only and dies off after a large part of the seeds are mature and have been dispersed.

According to Ellenberg and Leuschner (2010), *C. claviculata* belongs to the small group of central European liana-plants. The ability of climbing and winding is often found in plants with low Ellenberg continentality (=1) and high temperature values (=6) and in regions with long vegetation period, i.e. mild and short winters. The ability to climb enables the slender plant species to reach heights with better light conditions which may be advantageous in sites with well developed herb/grass layers.

The above ground dry biomass of plants sampled in four regions in central Europe (The Netherlands, north-west Germany, southern Sweden and north-eastern Germany) ranged from 0.04 to 32.5 g per individual, with a mean of 0.95 g (Table 2). Highest dry mass was found in populations from north-western Germany, whereas plants from north-eastern Germany were significantly smaller. These differences are most probably related to abiotic site conditions (lower soil moisture in north-eastern Germany) rather than related to genotypic differences. Measurements of the number of inflorescences are in line with the former results: north-eastern German individuals exhibited the lowest numbers (maximum 44 inflorescences) whereas the highest numbers were found in the more atlantic, western regions with 629 inflorescences in The Netherlands and 501 inflorescences in north-western Germany. Furthermore, north-eastern German individuals were shorter than those from the other regions. We measured a maximum of 83 cm in north-eastern Germany and a maximum of 196 cm in southern Sweden.

The capsules may contain between one and four seeds (see also chapter “Morphology”) but most capsules contain two seeds. However, in Spanish individuals we found a larger number of capsules with three seeds. Owing to constraints in resource allocation, seeds from Spain were lighter than those from all others regions.

Spatial distribution of plants within populations

In forest sites *C. claviculata* is found mostly in tens or hundreds of individuals. Here, the species occurs in small groups (Ellenberg *et al.* 1992, Voss personal observation). In contrast, populations in fringe communities or in places where the species has newly established may consist of only a few individuals which may stand very close to each other. It is often difficult to identify what is a single individual because the species tends to become interwoven with its neighbors. On forest clearings or other disturbed sites, individuals may occur in high densities of thousands of individuals, occupying an area of several tens or hundreds of square meters (Voss personal observation).

Phenology

Dierschke (1995) assigned *C. claviculata* to the symphenological *Sorbus aucuparia*-*Galium odoratum*-group which predominately flowers at the end of full springtime.

Usually the species is abloom between (May) June and September which is about two to three months after germination (e.g. Corillion 1983, Jäger and Werner 2005, Voss unpublished data). However, for Spain, a flowering period from March to October has been described (Castroviejo 1998). Boulenger (1914) noticed blooming individuals in England already at the end of February and Weeda (pers. comm.) observed flowering plants in The Netherlands in January. This opportunistic flowering is probably facilitated by the mild winter climate in these atlantic localities (e.g. du Buysson 1914). *C. claviculata* may survive mild winters abloom and overwintering plants die already in June (Griffioen 1961). This performance might be enabled by the sympodial shoot system (Fig. 2). In protected sites, e.g. under litter, shoots do not freeze and in spring the hibernating axillary buds cast out (Buttler 1986). However, this behavior is not just confined to mild winters. Even after the very long and cold winter in 2008/2009, we observed huge not blooming individuals in eastern Brandenburg and southern Sweden despite of temperatures of below -15° C. This phenomenon was also observed by Lethmate *et al.* (2002) for northern Northrhine Westfalia.

Reproduction

Like most other species in the tribe *Fumarieae*, *C. claviculata* is a synoecious, homogamic, self-compatible and autogamous species but it produces nectar and therefore is visited and pollinated by honey- and bumblebees (Lidén 1986, Klotz *et al.* 2002, Voss pers. observation).

The pollen is deposited on the stigma which is hidden between the apices of the inner petals. Flexible joints at the base of the inner petals allow them to be pressed down- or sideways. By this mechanism the stigma will be exposed to the visitor. After the visit the petals resume their original position immediately (Lidén 1986).

The gynoecium has 1-4 ovules (see chapter “Morphology”) and across the range there are on average 2.2 (± 0.1 s.e.) ovules per flower (Table 1). The androecium produces 489 (± 13) pollen grains which results in a pollen/ovule ratio of 236 (± 9), which is less than 10% of the pollen/ovule ratios of related outcrossing species of Papaveraceae (Erbar and Langlotz 2005) and thus suggests an autogamous breeding system (Michalski and Durka 2009). As the species contains on average two seeds per capsule only about 9 % of the ovules are aborted. An average individual may produce about 300 (21 inflorescences x 7 flowers/inflorescence x 2 seeds/capsule = 294) seeds (see Table 1 and chapter “Life cycle” for details on seed mass, number of capsules, seeds/capsule) but there may be also individuals with one single fruit or with almost 2000 seeds (Voss personal observation).

C. claviculata seeds possess an inconspicuous aril (see chapter “Morphology”). Often, an aril is considered as an adaptation to myrmecochory. Horstmann (2005) found nests of *Formica polyetena* Foerst. in a location of *C. claviculata*. We never directly observed that ants transported seeds of *C. claviculata*. However, in the presence of ants between 10 and 65 % of the seeds presented on the forest floor in petri-dishes (mammals and birds were excluded through small meshed cages) were removed after 36 hours (Voss unpublished data). These field observations suggest that for short distance dispersal (within populations) entomochorous dispersal might be of importance for the species. However, for longer distances anthropochory or epizoochory, i.e. unintended dispersal through humans and animals, respectively, are probably the most important dispersal mechanisms which may explain its strong spread during the past decades (chapter 2). There are neither own observations nor information on endozoochory dispersal of *C. claviculata*.

Germination

When the black, shiny seed of *C. claviculata* has imbibed water, the seed coat bursts open after a few days. One to two weeks later the hairy primary root appears. If sown on soil, the root is oriented straightly downwards into the soil. After further one or two weeks the two cotyledons are visible (Fig. 3).

In the family Papaveraceae many species exhibit a combination of morphological and physiological dormancy (=morphophysiological dormancy, Grushvitzky 1967). Probably this is also the case with *C. claviculata*: Buchenau (1861) described the embryo of *C. claviculata* as little differentiated at the time of dissemination (see chapter “Morphology”). This is in line with the observation that seeds germinated earliest two weeks after dissemination (see chapter “Life cycle”). Furthermore, at least for a large part of the seeds, cold stratification (cold pre-treatment) seems to be necessary in order to break physiological dormancy. In a climate chamber experiment with seeds from about 20 populations from each of the regions north-western Germany and southern Sweden, the impact of different stratification temperatures was tested. The seeds that we used in this and all following experiments described were harvested during a period of one month from parental plants which were cultivated in the greenhouse in order to minimize maternal effects. Seeds were slowly dried and stored for four weeks under dry conditions at room temperature before they were used in the first experiment. For the experiment 50 seeds per replication were put on moistened filter paper in a Petri dish. Germination was highest after stratification at 4°C (35.0±2.7%) compared to stratification at 0° (23.7±1.9) or 15°C (12.4±1.1; ANOVA: $F_{2,90}=43.58$; $p<0.001$; Chrzan *et al.* unpublished data). The results of this study are in line with the observation that the peak of germination occurs in spring after break of dormancy by low temperatures during winter. However, a small fraction of seeds does not need cold pre-treatment and thus, may already germinate in autumn, shortly after dissemination (see also chapter “Phenology”).

Additionally, in a comparison of stratification period lengths, we found that germination increased at an alternating temperature of 10/20°C with an increasing duration of the pre-treatment at 4°C. If we exposed the moistened Petri dishes for two or four weeks to the cold treatment, only 14.3 (±3.5) and 17 (±4.5)% of the seeds germinated, respectively, whereas there were about 20.2 (±4.5) and 20.8 (±4.6)% seed germination after six and eight weeks, respectively (ANOVA: $F_{3,40}=4.01$; $p=0.014$). However, an extension of the stratification period to more than 8 weeks did not increase germination. Generally, the majority of seedlings emerged four to six weeks after the beginning of stratification (Voss unpublished data).

Furthermore, we studied seed germination response to exposure to very low temperatures. We exposed seeds to a cold pre-treatment of -20°C and transferred them afterwards to a temperature of 4°C for germination. When the seeds were kept moist during stratification, 14.8±1.1% of the seeds emerged afterwards. Without cold pre-treatment there was a germination of 43.6±1.8% (LSD test; $p<0.05$; $n=20$). In contrast, when seeds were kept dry during stratification but moistened afterwards, germination was 50.9±1.7% and 50.2±1.2% with and without cold treatment, respectively (LSD test, not significant). Thus, very low temperatures by themselves do not damage seeds of *C. claviculata*, whereas imbibed seeds may be damaged through strong frost which, in turn, decreases germination success (Voss unpublished data).

Generally, seeds of *C. claviculata* may germinate in darkness as well as in daylight, though there was a tendency for an increased germination in daylight (18.3±2.0 vs. 20.1±2.1 %; ANOVA: $F_{1,64}=3.752$, $p=0.057$; Voss unpublished data).

In contrast, highest germination success (71±2% in petri-dishes, at 4°C) of *C. claviculata* was yielded with seeds which were not cultivated in the greenhouse but were collected in the field in north-western Germany (Table 1).

Seeds of *C. claviculata* mostly are spread together with the indehiscent pericarp (Fig. 3). In a study with *Ceratocapnos heterocarpa*, germination success of seeds without pericarp was about five times higher than germination success of intact fruits. Adding pericarp to naked seeds did not decrease germination, indicating that an indehiscent pericarp might impede germination physically but not through inhibiting chemical compounds. Moreover water and gas access to the seed was guaranteed by cracks or when the apical beak had fallen off. Thus, in the field, an indehiscent pericarp provides a greater chance of survival by delaying germination (Ruiz de Clavijo 1994). However, germination of seeds from *C. claviculata* was neither increased nor accelerated with pericarp compared to naked seeds (Buttler 1986).

There were no information available on seed bank type (Thompson *et al.* 1997, Kleyer *et al.* 2008). However, *C. claviculata* was found in soil seed banks in old British coppice woods. In total, 5.4 seeds per m² of soil cores from a depth of 0-15 cm germinated within two years (Brown and Oosterhuis 1981). We tested soil samples which were taken in six dense populations each in The Netherlands and north-western Germany. At a soil depth of 1-15 cm in total 41.2 seeds per m² germinated. Of course, these seeds may also have been transferred into this depth recently by burying animals or may have been accidentally translocated through the soil corer. Though we could find the species in the soil sample, the few existing studies do not present strong evidence for a persistent seed bank.

Response to competition and management

C. claviculata belongs to a group of forest species, which were avoided by game and even benefited from browsing animals through the creation of a less dense vegetation layer (Förster 1998). Moreover, game passes and resting places of wild boar not just create gaps in the vegetation layer but additionally disturb the soil surface and create optimum conditions for germination of *C. claviculata*. The strong increase of populations of wild boar during the past decades might have even reinforced this effect (Ellenberg and Leuschner 2010). Locations with intensive forestry management and clearfellings exhibit a similar disturbance but usually over much larger areas, and germination and establishment of ruderals and light-demanding species such as *C. claviculata* are promoted in these communities (Schulze-Motel 1986, Berg *et al.* 2004, Dengler *et al.* 2007).

In contrast, in locations with dense vegetation layer the species exhibits a very slender growth and the abundance of the species may be low. However, the tendrils enable partly an overgrowing of the surrounding vegetation and there, light conditions are better for growth of *C. claviculata* (Lethmate *et al.* 2002).

Herbivores and pathogens

The weevils (*Curculionidae*) *Procas granulicollis* Walton, also known as Climbing Corydalis weevil, and *Sirocalodes mixtus* Mulsant & Rey are feeding on *C. claviculata*. The former is found only in Great Britain and Spain (Thompson 2006). In Britain the nocturnal *P. granulicollis* often occurs in woodland clearings. According to Fowles (1992), *C. claviculata* is the sole recorded adult food plant of the weevil. It is leaving characteristic half-moon-shaped holes on the edge of the leaves. According to the EICA hypothesis (Blossey and Nötzold 1995) release of enemies such as predators or herbivores in the newly invaded range may enhance expansion process of species. However, although we observed feeding tracks of this form in several populations in the native range in The Netherlands and less often in all other study regions, we never observed neither of the weevils on the species (Voss unpublished data). Such damages can also be caused by slugs and caterpillars. A large number of caterpillars of the generalist species *Noctua pronuba* L. (Large Yellow Underwing) were found in a greenhouse cultivation of *C. claviculata* (Voss personal observation).

The thermophile, herbicole weevil *Sirocalodes mixtus* occurs from western Europe (but also in northern Germany, Austria and Turkey) to northern Africa predominantly on ruderal sites (Hoffmann 1954, Lohse 1983, Koch 1992). It was first reported in the Netherlands in 1993 and appeared to be common on *C. claviculata* and *Fumaria officinalis* (Heijermann and van den Berg 1995).

Furthermore, in cultivations and in populations in the Netherlands, infestation of the species by aphids could be observed (Voss personal observation).

Mycorrhiza

Very often species on acidic soils show mycorrhizal infection. So far, there is no information concerning mycorrhizal infection for *C. claviculata*.

Physiological data

Most of the seeds are dormant and will only germinate after cold stratification (see chapter “Germination”). Otherwise there is no information available on the physiology of the species.

Biochemical data

Papaveraceae are one of the few large plant families in which the formation and storage of phenylisoquinoline alkaloids could be detected in all analyzed genera (Hegnauer 1969). In many species (such as *Corydalis*, *Dicentra*, *Fumaria*, *Ceratocapnos*) alkaloids are bound to fumaric acid and are stored in the vacuole of alkaloid idioblasts (Hegnauer 1969).

The alkaloid chemistry strongly supports the subtribal classification of Fumariae. The compounds protopine, sanguinarine and coptisine are very common and they have the status of subfamily characteristics in Fumarioideae; the compound cularine is only found in this subfamily (Lidén 1993, Nuhn 2006). Apart from alkaloids, in this subfamily the flavonoid and aminoacid delta-acetylornithin could be detected which may serve as a transport form of nitrogen. Furthermore small amounts of arginine and glutamine are present (Hegnauer 1969).

Up to now, more than 50 alkaloids could be detected in *C. claviculata* (Table 4). It has the following compounds in common with the closely related genus *Sarcocapnos*: celtin, celtisine, breoganine, sarcocapnidine, oxosarcocapnidine, calviculine, sarcocapnine, oxosarcocapnine, crassifoline, 4-Hydroxycularine, limousamine, ribasin and ribasidine (Hegnauer 1969, Boente *et al.* 1983a). In *C. claviculata* the alkaloid fraction consists mainly of cularine, cularidine and cularicine whose occurrence is limited to Papaveraceae (Blaschke and Scriba 1985). Cularine is described as a relaxant against contractions in the human bronchus thus having antispasmodic activities (Candenat *et al.* 1990). Furthermore it may produce anesthesia of the cornea, a drop in blood pressure, an increase of heart tone and heart contractility, it has an effect on smooth muscles by stimulating the uterus and depressing the intestinal motility (Reynolds 1940, Blaschke and Scriba 1985). Finally cularines possess a similar arrangement of the nitrogen-oxygen-oxygen bonds as found in antineoplastic agents and thus they might have a similar impact (Castedo and Suau 1986). Cularidine (and also reticuline) work inhibitory on smooth muscles and due to this cause vasorelaxation (D'Ocon *et al.* 1991, Paulo *et al.* 1992). Some of the alkaloids in *C. claviculata* like crassifoline or cularine serve only or partly as precursors for other alkaloidic compounds like canentrine or noyaine (Boente *et al.* 1983b, Blaschke and Scriba 1985). In general, isoquinoline compounds account for resistance against phytopathogenic organisms and many of them are pharmacologically active for which reason they were already used in ancient times as antiseptics, analgetics, antispasmodics, sedatives and laxatives (Blaschke and Scriba 1985, Hao and Quicheng 1986, Kuo *et al.* 2004). Some of the protoberberines (berberine, coptisine, protopine, scoulerine and stylopine), dihydrosanguinarine and isoboldine have cytotoxic activities and thus may have antiulcer, antibacterial or antifungal activity.

Table 4. Alkaloidic groups and compounds found in *Ceratocapnos claviculata* (Boente *et al.* 1983a, b, Boente *et al.* 1984a, b, Scriba 1984, Boente *et al.* 1986a, b, Castedo and Suau 1986, Allais and Guinaudeau 1990).

Alkaloidic group	Compound
Aminoethylstilbene	crassifoline Methine, Leonticine (=petaline Methine)
Aporphine	(+)-Isoboldine, Thaliporphine
Benzophenanthridine	Dihydrosanguinarine
Benzyltetrahydroisoquinoline	(+)-Crassifoline, (+)-Juziphine, (+)-Reticuline
Cancentrine	(+)-Claviculine, Oxosarcocapnidine, (+)-Sarcocapnidine, Sarcocapnine, Oxosarcocapnine
Cularine	Breoganine, Celtine, Celtisine, (+)-Corycularicine, (+)-Culacorine, (+)-Cularicine, O-Methylcularicine, Oxocularicine, (+)-Norcularicine, (+)-Cularidine, (+)-Norcularidine, Cularimine, (+)-Cularine, Oxocularine, (+)-Enneaphylline, Sauvagnine, Dihydrosauvagnine, Secocularidine
Dibenzazonine	Crassifolazonine
Dibenzofuranazepine	Clavizepine,
4-Hydroxycularinalkaloids	Hydroxycularine, (+)-Limousamine, Hydroxysarcocapnine
Indanobenzazepine	(+)-Ribasine [= (+)-Limogine], (+)-Norribasine, Ribasidine
Morphinanedienone	(-)-Pallidine
N-Benzyltetrahydroisoquinoline	Viguine
Proaporphine	(-)-Glaziovine
Protoberberine	Berberine, (-)-Cheilanthifoline, Coptisine, 8-Oxycoptisine, (-)/(+)-Stylophine, (-)-Scoulerine, (+)-Protopine
Secocularine	Norsecocularine, Noyaine

Furthermore, some of them were detected to contain chemicals which may work as defense against arthropods (Leitao da-Cunha *et al.* 2005, Veldman *et al.* 2007, Vrba *et al.* 2009).

Interestingly, the compounds culacorine, norcularicine and oxocularine could only be isolated in individuals of *C. claviculata* from the surroundings of Limoges by a French group (Allais and Guinaudeau 1983) but were not found in collected plants from the surroundings of Bremen by Blaschke and Scriba (1985). On the other hand the compounds claviculine, crassifoline, cularimine and sarcocapnidine were described in German but not in French specimens. These differences might be due to measuring inaccuracies but according to Blaschke and Scriba (1985) also chemical races might be the cause of such differences.

Genetic data

Like in *Ceratocapnos heterocarpa*, the chromosome number of *C. claviculata* is $2n=32$ (Reese 1951, Ryberg 1960, Gadella and Kliphuis 1966, Queiros 1981, Lidén 1986, Elena-Rosselló 1987). Assuming a basic chromosome number of $x=8$, the species is tetraploid (Klotz *et al.* 2002). However, in a single literature source, in addition also $2n=64$ was mentioned (Queiros 1981). In *Fumarieae* chromosomes have a length of less than $1\ \mu\text{m}$. In *Corydaleae* each chromosome measures about $2\ \mu\text{m}$ which is in line with inbreeding annuals usually having smaller chromosomes than outbreeding perennials (Lidén 1986).

Hybrids

As there are no close relatives across the current range there is no information on hybridization. However, in the tribe *Fumarieae* almost all species have strong intersterility barriers (Lidén 1986).

Status of the species

Across the entire range, the species is neither economically used nor under protection. In fact, during the last four decades a spread within its native range such as in the UK (Hill *et al.* 2004), in northern Belgium (Vannerom 1994) and north-western Germany (Pott and Hüppe 1991, Pott 1990, Jäger and

Werner 2005) has been noted. Furthermore, the species showed also a strong expansion eastwards- and northwards towards suboceanic and north-temperate regions and established a number of populations in northern France (Decocq 2000), in north-eastern North Rhine Westphalia (Pollmann and Lethmate 2006), Mecklenburg-Western Pomerania (Meyer and Voigtländer 1996), north-eastern Lower Saxony (Dengler 2007), north-western Saxony-Anhalt (Passarge and Hofmann 1968, Rattey 1984) and Brandenburg (Benkert *et al.* 1995) in Germany, as well as in Skåne and Blekinge in southern Sweden (Hylander 1971, Oredsson 2005). Occasional populations are also found in north-western Bavaria, Rhineland Palatinate, Hesse, eastern Thuringia, Saxony (Jäger and Werner 2005) and Austria (Kleesadl 2009).

A common character of all these sites is that they originally have been acidic beech or birch-oak forests but now changed to rather non-natural (e.g. stands of *Pseudotsuga*, *Pinus*, *Larix*, *Robinia*) or non-constant forest communities, clearings or disturbed sites (Pott 1995, Benkert *et al.* 1996, Pollmann and Lethmate 2006, Ellenberg and Leuschner 2010). Additionally, the species occurred in most of the sites spontaneously, often with distances of 10s to 100s km from the next population and in higher abundance than in native range sites (Rattey 1984 and chapter 2 Fig. 1).

Possible causes for the successful establishment might be increased nitrogen depositions and soil acidification and, especially towards the east, milder winters of the last decades (shift of the 0°C isoline towards north-east) due to global change (Bobbink *et al.* 2010, Pott 1990, Decocq 2000, Lethmate *et al.* 2002, Parmesan and Yohe 2003). However, this alone does not explain the fast expansion over long distances and occurrence of isolated populations. Thus, direct anthropogenic activities such as transport of wood and forest saplings from tree nurseries, afforestation and clearcutting in the course of intensive forest management, and disturbance of the vegetation layer and soil surface might have the largest influence on successful dispersal and establishment of the therophytic species (Buttler 1986, Decocq 2000, Oredsson 2005, chapter 2 and see also this chapter “Reproduction”). E.g. occurrences in Sweden might be related to seeds or fruits which were attached to timber bark and which was transported as pulpwood and sawtimber to Swedish sawmills and paper plants after severe storms in north-western Germany and The Netherlands in the 70s (Buckley *et al.* 2003, Oredsson 2005). Once introduced, secondary factors such as atmospheric nitrogen deposition or milder winters may facilitate successful establishment of the species.

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4. Homesite advantage and phenotypic plasticity in a range expanding forest therophyte

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In preparation

Abstract

Local adaptation resulting in homesite advantage and phenotypic plasticity in fitness relevant traits may be important factors for successful colonization and establishment of therophytic plant species. In this study we tried to identify these both evolutionary strategies in the native and in the invaded range of the expanding eu-oceanic *Ceratocarpus claviculata*. Additionally, we studied the potential for further expansion of the species.

Plasticity was studied in a factorial field experiment with seeds from each of two regions in the native and in the invaded range for the factors sun exposition and nitrogen. A reciprocal seeding experiment in each of the two regions in the native and invaded range served to study presence and effects of homesite advantage and potential for further expansion.

In the common garden experiment, we found differences between seeds of native and neophytic origin (germination $p < 0.001$, length $p < 0.05$, capsules per inflorescences $p < 0.001$), indicating genetic differences. Local adaptation might be indicated by interactions between the factors origin and habitat quality for various traits. However, *C. claviculata* showed also phenotypic plasticity towards the factors nitrogen availability and sun exposure.

In the field experiment there was no difference in establishment success (n.s.) between all origins. Native seeding regions featured higher germination ($p < 0.001$) and establishment ($p = 0.002$) than neophytic regions. We detected homesite advantage for seeds of native origin with respect to germination and plant length and for native and invaded range with respect to establishment. Future expansion into slightly more continental region seems improbable.

Despite very low genetic variation and thus lacking evolutionary potential *C. claviculata* expanded successfully its range probably through general purpose genotypes and phenotypic plasticity. In contrast, differences between origins and homesite advantage in some fitness relevant traits indicate at least some adaptation to local environmental conditions during colonization process. However, still there is higher germination and establishment in the native range which may be related to more favorable environmental conditions such as nutrient availability, soil moisture, climate and floristic composition.

Keywords

global change, *Ceratocarpus claviculata*, range expansion, autogamous, therophyte, local adaptation, reciprocal seeding

Introduction

During range shifts and expansions a number of environmental filters as well as species traits determine the outcome of colonization events (Barrett *et al.* 2008, Lortie *et al.* 2004, Fenner and Thompson 2005). Species traits may be linked with the evolutionary potential of the species through high phenotypic plasticity or local adaptation to habitat conditions and selection of particular phenotypes (Jump and Peñuelas 2005, Linhart and Grant 1996).

Local adaptation implies that populations of a species often evolve traits that are beneficial under local environmental conditions but which may not be beneficial under other conditions (Kawecki and Ebert 2004). This may be reflected by e.g. optimized flowering time and a corresponding higher fitness in the centre of the range compared to beyond the current range (Atkins and Travis 2010, Sagarin *et al.* 2006 and references therein). Thus, within their range, populations should adapt sufficiently due to the existing selection pressure through abiotic (edaphic, climatic) or biotic (herbivores, pathogens, mutualists) habitat conditions (Joshi *et al.* 2001).

Extension of the species range border involves that the border populations adapt or are preadapted to the local conditions at and beyond the range border. Then the species may invade into new sites and has a higher chance to establish successfully (Angert and Schemske 2005, Scott *et al.* 2010). Thus, the process of local adaptation plays an important role in determining the dynamic of range shifts under environmental changes (e.g. Atkins and Travis 2010).

In fact, individuals of populations at the edge of a species range or in newly invaded range sites may sometimes even show a homesite advantage (as result of local adaptation) in fitness relevant traits in comparison to introduced congeners from the centre of the range. Thus, populations may feature changes in life history traits suggesting adaptations of local genotypes to the new habitat conditions (Santamaría *et al.* 2003). There are numerous examples of homesite advantage at or beyond the range border for traits such as time of flowering and thermal tolerance (Atkins and Travis 2010), tolerance to shade (Godoy *et al.* 2011), to different soils (Petrů and Tielbörger 2008, but see Macel *et al.* 2007) or evolution of increased competitive ability (EICA) after the release of enemies (Keane and Crawley 2002). In contrast, phylogenetic history (gene flow, drift) sometimes may explain a comparatively small part of recent local adaptation in fitness related characters (Joshi *et al.* 2001).

A prerequisite for local adaptation is the existence of genetic differentiation between different regions (Jakobsson and Dinnetz, 2005). Isolation of a population and large population size may foster differentiation because isolation prevents gene flow which would reduce the frequency of locally favoured and probably fitter genes (Holt and Golumkiewicz, 1997). In turn, large population size may ascertain genetic variation and thus, evolutionary potential through an increased ability to adapt to local habitat conditions or environmental changes (Holt and Golumkiewicz, 1997, Ouborg and van Treuren 1994).

An alternative, not mutually exclusive but complementing strategy to establish successfully in a wide range of habitats represents phenotypic plasticity which may be defined as a range of phenotypes of a given trait a single genotype can express as response to an environmental stimulus (Nicotra *et al.* 2010). E.g. plant species with a wide spatial or ecological distribution (e.g. *Lotus corniculatus*: Macel *et al.* 2007, *Mahonia aquifolium*: Ross *et al.* 2009, aquatic plants: Santamaría *et al.* 2003) often exhibit high gene flow and/or limited genetic variation (Nicotra *et al.* 2010). In these species, range wide high fitness may be assured by the occurrence of a general purpose genotype which is able to maintain a high fitness over a broad range of environmental conditions through compensatory plastic response in morphology and physiology (Schlichting 1986). These properties also characterize “ideal weeds” (Baker 1965). General purpose genotypes and plasticity allow introduced species to naturalize across a

range of habitats. Furthermore, they could have a fitness advantage in newly invaded founder populations where local adaptation has not occurred yet because of a lack of genetic variation (Bossdorf *et al.* 2005). Bossdorf *et al.* (2005), comparing phenotypic plasticity between native and invaded populations in a meta-analysis, found that in five of ten studies the latter were more plastic. This was interpreted as examples of adaptive post invasion evolution.

Beside the genetic predisposition and adaptive evolutionary processes, macroclimate and the availability of suitable microsites play an important role for successful establishment. Habitat requirements, such as contact to substrate, light, temperature, water, nutrients and competition, must be more or less met to ensure persistence of populations. (e.g. Price and Morgan 2006). Especially in therophytic species successful germination and establishment are the key stages in the life cycle (Fenner and Thompson 2005) that are strongly controlled by abiotic factors.

In former studies, local adaptation was often studied with respect to introduction and genetic reinforcement of individuals from other populations or establishment of new populations to prevent extinction of rare plant species (e.g. Becker *et al.* 2006, Bowman *et al.* 2008, Leiss and Müller-Schärer 2001, Vergeer *et al.* 2004) or with respect to invasions of non-native species (e.g. Verhoeven *et al.* 2011 and therein). Here, we study the range expansion of the self-pollinated, therophytic *Ceratocarpus claviculata*. During the last 30 years a gradual and spontaneous long distance spread of between 10s and 100s of km towards east and north beyond the original distribution range as well as within the range was noted. The original range of the eu-oceanic species was mainly in western Europe. Here it is growing on acidic, humic soils in open beech and oak-birch forests, forest clearings and pine plantations (Ellenberg 1991, Jäger and Werner 2005, Schultze-Motel 1986). In the more continental neophytic range, *C. claviculata* occurs mostly in substitute communities such as pine forests or on sun exposed forest clearings. Possible causes for the long distance dispersal may be direct anthropogenic activities such as wood transport and habitat disturbance through intensive forest management (chapter 3). However, once introduced it remains unclear how *C. claviculata* could establish successfully in those suboceanic and north-temperate regions. Ongoing postglacial recolonization processes might be related to the observed current expansion into the neophytic range (chapter 3). Alternatively (or additionally), it has been suggested that increased nitrogen depositions combined with soil acidification and milder winter during the past decades due to global change might be important factors that facilitated range expansion (Bobbink *et al.* 2010, Pott 1990, Decocq 2000, Lethmate *et al.* 2002, Parmesan and Yohe 2003).

In order to understand the range expansion of *C. claviculata* our study addressed the following questions:

1. Do individuals originating from the native and invaded range feature similar responses when grown under similar environmental conditions? Is it possible to identify local adaptation/homesite advantage of *C. claviculata* to the native and invaded range site or does the species rather show phenotypic plasticity in response to changing environmental conditions?
2. Can *C. claviculata* establish beyond the current range margin?

We studied phenotypic plasticity of *C. claviculata* for the factors sun exposition and nitrogen in seeds/plants from two regions in each of the native range and the neophytic range in a factorial common garden experiment. Additionally, we carried out a reciprocal seeding experiment in two regions in each of the native range and the neophytic range to test for presence and effects of local adaptation and climatic factors on establishment and fitness. The impact of environmental and genetic variation to phenotypic plasticity can be assessed in reciprocal-transplant experiments through significant home vs. away differentials which are assumed to reflect local adaptation (Joshi *et al.*, 2001). Finally, in order to draw conclusions about potential future expansion processes and to detect

climatic limitations for establishment we set up plots in non-invaded regions beyond the current range of the species. In the respective seeding regions we studied also biotic and abiotic factors to assess habitat quality and habitat requirements of the species.

Material and Methods

Study species

The biology of the tetraploid *C. claviculata* (L.) Lidén (Fumariaceae) has been reviewed recently (chapter 3). Its small flowers may be pollinated by insects (Apidae, Bombyliidae) but selfing due to autonomous self-pollination does often occur (Klotz *et al.* 2002, Lidén 1986). The main flowering time lasts from June to September. An average individual produces about 300 black, shiny seeds of 1.3 mg weight. Seeds have an inconspicuous, tiny aril and lack appendages for animal or wind dispersal. Thus, under natural conditions long distance seed dispersal should be rare. *C. claviculata* may germinate either in autumn or in spring and thus is winter as well as summer annual.

Study sites and seed collection

We chose four regions, eastern Netherlands (NL), north-western Germany (NWG), north-eastern Germany (NEG) and southern Sweden (S or S1 when compared with the other Swedish sites S2 and S3), of which two (NL and NWG) were situated within the original range and two (NEG and S1) within the neophytic range. In each region we chose five sites in which we set up the study plots. The regions cover a temperature and continentality gradient (Table 1) with regions NL and NWG situated in the atlantic zone and NEG and S1 in the sub-continental and northern-temperate zone.

Additionally, we carried out the seeding experiment at two sites outside the current range in order to test whether the realized and the potential range of the species differ and to assess whether the species' expansion has come to equilibrium. These two sites were situated along a continentality gradient near Ljungby (S2) and Värnamo (S3, both in Sweden) and differed from the invaded region S1 (Gualöv) by a difference of ca. -1°C and -2°C, respectively, of mean annual temperature (SMHI, 2010 and Table 1). For logistic reasons in each of the two regions outside the range we chose only two sites in coniferous forests where we placed five plots each. The further set up was equivalent to the reciprocal seeding experiment.

For a general description of all seeding regions, vegetation cover (herbs and bryophytes) was estimated in all study sites in each of the six regions. We set up one to three vegetation relevés according to homogeneity of the herb layer in the respective site, resulting in 48 relevés in total. Species in the shrub and tree layer were excluded from the analysis because many of the stands were forest plantations and the tree and shrub layer may thus not reflect the environmental site conditions properly. To avoid the undue influence of rare species we removed species that occurred in less than 3% of the relevés. Using the cover values, we calculated the weighted mean Ellenberg indicator value (Ellenberg 1992) for each relevé.

Instead of using seedlings which are common in transplant experiments, we used seeds which give us the possibility to study effects on the entire life cycle of the species (Scott *et al.* 2010). We collected seeds of 15 individuals each of 10 populations in each region randomly in a transect line. With this design we intended to compensate fitness differences of the individuals due to differing habitat quality. For logistic reasons we did not use seeds from one genotype. However, genotyping analyses had shown low genetic variation within populations and regions (chapter 5). Thus, we could assume that different responses for different factor levels of sun exposition and nitrogen would indicate phenotypic plasticity. In order to avoid maternal effects (Roach and Wulf 1987), we stratified the field-collected seeds at 4°C and germinated these in a greenhouse (substrate: Fruhstorfer Erde Typ P,

Hawita-Gruppe, Vechta). We harvested the seeds of this F1-generation and prepared mixed seed samples of each region for the experiment. The F1-seeds were harvested during a period of one month, air dried and stored for four weeks under dry conditions at room temperature before they were used in the experiments.

Table 1. Mean temperature from the coldest months January-February ($T_{\text{Jan-Feb}}$), the main vegetation period from March to July ($T_{\text{Mar-Jul}}$), mean precipitation per month from March to July ($\text{Prec/month}_{\text{Mar-Jul}}$) and mean number of days with precipitation per month from March to July ($\text{Days Prec}_{\text{Mar-Jul}}$) for the period 1961-1990 and 1990-2009 (DWD 2011, SMHI 2011).

Region	Climate station	$T_{\text{Jan-Feb}}$ (°C)		$T_{\text{Mar-Jul}}$ (°C)		Prec/Month _{Mar-Jul} (mm)		Days Prec _{Mar-Jul}	
		1961-1990	1991-2009	1961-1990	1991-2009	1961-1990	1991-2009	1961-1990	1991-2009
NL	Nordhorn	1.8	3.1	11.6	12.9	65.2	67.5	14.3	14.4
NWG	Bremen	1.1	2.6	11.5	12.4	59.0	60.4	15.7	15.0
NEG	Lindenberg	-0.7	1.0	11.8	12.9	50.0	52.2	13.6	13.6
S1	Kristianstad	-1.0	0.6	9.9	10.6	44.9	48.6	12.5	11.1
S2	LjungbyD	-2.6	-0.7	9.1	10.0	55.1	67.2	16.5	16.4
S3	Värnamo, Haghult, Kävsjö	-3.6	-2.1	8.4	9.2	55.1	68.4	16.6	16.3

Reciprocal Seeding experiment

In August 2009 (corresponds approximately to the time of natural seed shedding), we established study plots in five sites within each region (except S2 and S3, see above). The selected sites should be free from *C. claviculata* plants and seeds but at the same time appropriate for the species. Therefore, we selected sites situated at the edge of extant *C. claviculata* populations and with similar understory vegetation like sites harboring the species. In each population there were three random replicate rows (five in S2 and S3) of five plots, resulting in a total of 15 plots per region. Each row harbored one plot per region of seed origin plus one control plot without seeds. Regions of origin were randomized in a row with a distance between plots of 50 cm. We used 100 seeds per plot (50*50 cm²). Plots were not further prepared for the experiment. Response variables were: relative germination in October 2009 ($\text{Seedlings}_{\text{October}}/100$) and April 2010 ($\text{Seedlings}_{\text{April}}/(100-\text{Seedlings}_{\text{October}})$), number of established adults (we counted all seed shedding individuals per plot), shoot length and number of fruits. Length and number of fruits of individuals which were collected in July 2010 could not be determined in all adult individuals because in some places the individuals had already dried. Due to the slender, hygrophilic growth, plants broke into fragments during collection and thus the proper estimation of length/number of fruits was impossible. The experiment consisted of the two factors region of origin (=origin) and seeding region (=region) each with four factor levels (NL, NEG, NWG and S).

In addition, we recorded plot characteristics such as cover of mosses, litter, herb and tree layer in percent and light conditions (full shadow, half shadow). Plots were visited three times after the sowing in July 2009: in October 2009 and April 2010 in order to count seedlings germinating in autumn and spring, respectively, and in June 2010 to count adult, fruiting individuals, which served as a measure for successful establishment. Soil samples were taken from each plot, mixed to a soil sample per each 5-plots-row to estimate C/N ratio as a potential for nutrient mineralization. Total carbon and nitrogen concentration of the ground plant tissue was determined using an elemental analyzer (CE instruments, EA 1110, Italy; CE Instruments, 1996).

Factorial common garden experiment

We analyzed the effects of founder (seed) origin (NL, NWG, S, NEG) and habitat quality (factors: sun exposure and nitrogen availability) on the establishment of *C. claviculata*. For the factor founder origin, 50 seeds of a mixed seed sample from each of the origins NWG, NL, NEG and S were used per

replicate. The factor habitat quality (environmental conditions) consisted of a combination of two levels of nitrogen availability and two levels of sun exposure. Nitrogen availability consisted of the levels 5 (=N0) and 50 kg nitrate (=N+) deposition per ha and year, thus representing a site with low atmospheric nitrogen deposition and a site with high deposition, respectively (EMEP - European monitoring and evaluation program, <http://www.emep.int/>). The soil (Fruhstorfer Erde Typ P, Hawita-Gruppe, Vechta) that we used in the experiment contained 160 mg/l nitrogen. We found the species in a wide range of habitats under different light conditions. Thus, we tested additionally the effect of sun exposure by exposing the pots to the sunlight (=L+) which should mimic situations in treeless open areas. Alternatively, the pots were put under cages with gauze, corresponding to situations in open forests (=L0) which is equal to a light quantity of approximately 70%. Thus, we created four environments that represented a range in habitat quality from nutrient-rich open sites to nutrient-poor shaded site: N+L+, N+L0, N0L+ and N0L0. For each environment we used twelve replicate pots (only for the response variable germination). After seedling emergence, we transplanted four of the seedlings to pots with a volume of 3 l. If there were less than four seedlings, we transplanted as many as there were available. Soil volume and nitrogen application corresponded to the number of individuals per pot. All pots were watered every second day. Thus, shaded pots were kept moist. However, due to higher energy input sun-exposed pots fell intermittently dry. We measured the response traits germination, shoot length and total biomass of the shoots of the plants in one pot, specific leaf area (SLA), number of inflorescences, capsules per inflorescence, seeds per capsule and seed mass.

Statistical analysis

The analyses were carried out with CANOCO (partial DCA; ter Braak and Šmilauer 2002) and STATISTICA 10 (ANOVA, HSD-test, LSD-test, linear regression; StatSoft Inc, 2011).

In the reciprocal seeding experiment, we performed a factorial ANOVA for nested designs testing for the effects of seeding region, seed origin and seeding site (nested in seeding region) on germination, establishment, length and number of fruits. If ANOVA was statistically significant for the effect of an environmental factor (here the different seeding regions) this was interpreted as differences in fitness (or as plasticity of the analyzed trait) under different environmental conditions. In case of local adaptation/genetic differentiation we expected that plants from different regions of origin showed different responses to different habitats (=seeding regions). This genetic variation in a trait should be indicated by a region×origin interaction and a higher fitness of individuals/seeds in the region of their origin as compared to seeding regions or a higher fitness of seeds of an origin in their homesite compared to other seeds (homesite advantage). If there was only a significant effect of seeding region then all origins responded similarly plastic under a changed habitat. If there was only a significance of region of origin but no plastic response under changed habitats this might indicate genetic differentiation between the origins or, in case of homesite advantage, non-plastic local adaptation to the region of origin (Nicotra *et al.* 2010).

We used LSD test for equal sample size and HSD test for unequal sample size as post hoc test to compare response variables between seed origins and seeding regions. A “home-vs.-away” contrast of the “origin” × “seeding region” interaction served to test for local adaptation (homesite advantage). This was carried out for all four homesite combinations (one combination was e.g. the outcome of seeds of the origin NWG in the seeding region NWG was tested vs. all other seed origins in the seeding region NWG). Alternatively, a “home-vs.-away” contrast of the “origin” × “seeding region” interaction was only carried out for the seeds from the native range regions (NWG, NL). We did these two alternatives owing to the assumption that individuals in the newly invaded sites have not evolved local adaptations yet.

Due to strong intercorrelation of the predictor variables (percentage cover of the plots) we did not carry out multiple regression but used several linear regression analyses to test whether germination was related to one of the variables. Whenever data did not meet assumptions of normality it was arcsinus-square-root (germination, establishment) or Box-Cox-transformed (length, number of fruits) before analysis. Factorial ANOVA and a native vs. invaded contrast were used to test for differences in habitat quality (C/N, Ellenberg indicator value) between the seeding regions. In order to visualize the relationships among vegetation plots and to account for the effects of varying plot size in the data we run partial DCA ordination with species cover abundance data. We applied detrending by 2nd order polynomials (ter Braak and Šmilauer 2002) and used “relevé size” as covariate. For interpretation purposes, we overlaid mean Ellenberg indicator values which were calculated for each plot.

In the common garden experiment, the response variable germination was arcsinus-square-root-transformed and SLA was Box-Cox transformed before analysis. In order to consider the unequal number of individuals per pot (ranging between one and four individuals) we carried out linear regressions with all response variables and the number of individuals per pot. In case of significant correlation, we used the residuals of the analyses as input values for the subsequent factorial ANOVA and tested for the effects of seed origin (four levels) and habitat quality (four levels). We used HSD test for unequal sample size to compare the four treatment combinations and the different seed origins. Contrasts served for a comparison between native and invaded regions of seed origin in order to test for adaptations to the invaded range. Here a studied trait (e.g. SLA) was considered plastic in *C. claviculata*, if the test for the effect of habitat quality (e.g. sun exposition) on that trait was statistically significant. If seed material from different regions of origin shows different responses under changed habitat quality (significant “origin”×”habitat quality” interaction) we assumed adaptation to different local habitats. If there was only a significance of origins but no plastic response under changed habitat quality we interpreted this as genetic differentiation between the regions of origin (Nicoltra *et al.* 2010).

Results

Reciprocal seeding experiment

We yielded a germination of 0.5% ($\pm 0.1\%$ s.e., $n = 240$) in October 2009 (winter annual), 12% ($\pm 0.7\%$ s.e.) in April 2010 (summer annual) and an absolute establishment until July 2010 of 3.7% ($\pm 0.5\%$ s.e.) of the originally sown seeds.

Table 2. Results of nested ANOVA for the response variables germination in October 2009 and April 2010, establishment, shoot length and number of fruits until July 2010. Values were transformed prior analysis. Region and origin are fixed factors; site is random factor, $n=240$.

	df	October			April			July			Length			# Fruits		
		<i>F</i>	<i>p</i>		<i>F</i>	<i>p</i>		<i>F</i>	<i>p</i>		<i>F</i>	<i>p</i>		<i>F</i>	<i>p</i>	
Region	3	1.286	ns		1.170	ns		0.55	ns		3	9.590	<0.001	3	10.675	<0.001
Site (Region)	16	8.743	<0.001		18.556	<0.001		40.46	<0.001		15	11.536	<0.001	11	3.156	0.002
Origin	3	21.330	<0.001		16.974	<0.001		1.70	ns		3	0.100	ns	3	0.234	ns
Region x Origin	9	3.088	0.002		2.370	0.014		1.27	ns		9	1.079	ns	8	1.171	ns
Home vs. away	1	2.804	ns		3.129	ns		7.73	0.006		1	0.897	ns	1	0.540	ns
Region: Native vs. invaded	1	3.973	0.047		56.789	<0.001		9.92	0.002		1	23.11	<0.001	1	0.774	ns

Generally, seeds of the origin S and NWG germinated better than those of the other two origins (both $p < 0.001$, Fig 1, Table 2) indicating genetic differentiation of *C. claviculata* between origins. However, this difference was no more significant for adult individuals in July ($p > 0.05$). The response variables length and number of fruits were not related to origin (Table 2 and 3). Differences in habitat quality between seeding regions were not significant in the nested ANOVA but the post hoc tests and the contrast native vs. invaded showed an advantage of the native range with respect to germination and establishment ($p_{Oct} < 0.05$, $p_{Apr} < 0.001$, $p_{Jul} < 0.01$, Fig.1, Table 2). Additionally, other fitness parameters such as length and number of fruits were highest in NWG and lowest in NEG (Table 3).

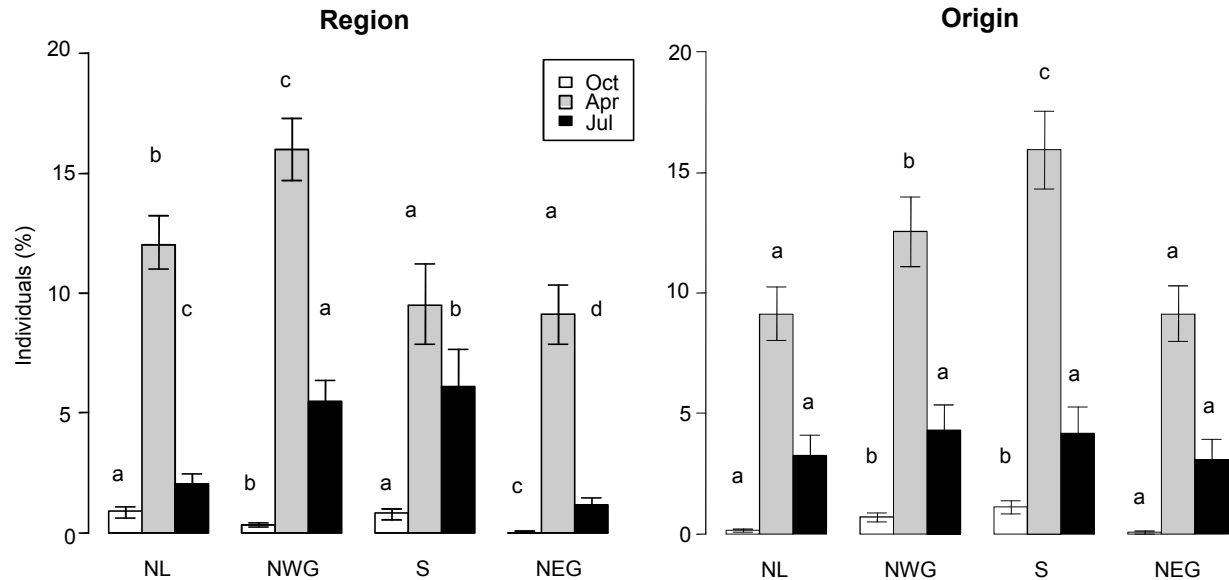


Fig. 1. Germination in October 2009 (Oct) and April 2010 (Apr) and established individuals in July 2010 (Jul) shown as Individuals (%) in the four sowing regions (Region) and of the four seed origins (Origin). Barplots show the means calculated of all samples over one region or one origin, respectively, error bars show the standard errors. Letters a-d indicate significant groups for each time of data record (LSD test), $n=240$.

However, establishment and number of produced fruits were as high on Swedish as on north-western German sites (Fig 1 Region, Table 3). We detected a homesite advantage for the seeds of native origin with respect to germination in April and length ($p < 0.001$, not shown) and for native and invaded origin with respect to established individuals in July (Fig. 2, Table 2).

In contrast to the seeding experiment, we found climatic and floristic differences between seeding regions which may indicate differences in habitat quality as shown by Ellenberg indicator values. However, NWG and S featured high floristic similarity. In the invaded range 80-100% of the sites were half shaded, whereas in the native range 80-86% of the sites were found in fully shaded situations (data not shown). This is in line with the tree cover of the plots (42.5 and 39.6 % in native vs. 34.5 and 26.9 % in invaded range, $p < 0.001$) and with Ellenberg IV for light in NL (Fig. 3). Generally, sites in the native regions feature a slightly better nutrient supply as C/N ratios ranged between 23.6 (NL) and 26.3 (NEG, $p < 0.001$; native vs. invaded range $p < 0.001$) and as corroborated by the Ellenberg indicator value for nitrogen (Fig. 3). Ellenberg indicator value for soil moisture was higher in the native than in the invaded range (Fig. 3).

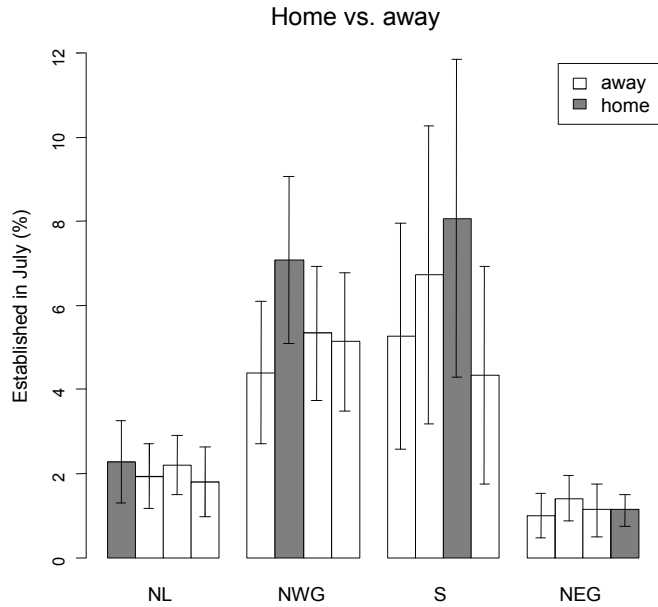


Fig. 2. Established individuals in July (%) of all origins in all regions (interaction between region and origin). Individuals with seed origin in the respective sowing region are referred to as “home” all others as “away”. Means are calculated of each origin-region-combination.

0.13, $p < 0.001$, not shown), on sites with higher tree cover ($r^2 = 0.08$, $p < 0.001$ and $r^2 = 0.03$, $p < 0.001$) and on sites with increasing litter layer ($r^2 = 0.14$ and 0.11 , $p < 0.001$). In contrast, there was a negative correlation between germination and establishment success and cover of the herb layer ($r^2 = 0.12$ and 0.09 , $p < 0.001$), and cover of bryophytes ($r^2 = 0.05$ and 0.06 , $p < 0.001$).

In the two regions beyond the current range (S2 and S3, Fig. 3), habitat conditions were more continental, more sun-exposed and soil featured lower moisture than the successfully invaded south of Sweden (S1). Seedling emergence (28 of a total of 16000 seeds = 0.18%) was much lower than in the native and newly invaded range and only 0.025% (4 of 16000 seeds) of the seedlings established successfully (data not shown).

Table 3. Length and number of fruits (# Fruits). Shown are mean, standard error (s.e.) and total number of individuals (n) for the different regions and origins. Letters a-c indicate significant groups (HSD test $p < 0.05$).

		NL		NWG		NEG		S		Total n
		Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	
Length	Region	17.22 ^b	2.19	29.14 ^c	3.42	8.54 ^a	0.64	14.38 ^{ab}	2.51	134
	Origin	21.66 ^a	3.26	18.15 ^a	2.54	17.16 ^a	2.45	18.21 ^a	3.66	134
#Fruits	Region	8.00 ^a	1.70	38.37 ^b	11.48	2.46 ^a	0.61	20.67 ^b	3.67	85
	Origin	16.75 ^a	4.91	21.52 ^a	5.37	16.86 ^a	4.98	30.13 ^a	16.95	85

This corresponds to higher mean precipitation and a higher number of rainy days in the native range (Table 1). Similarly, indicator value for continentality increased and temperature decreased from west to east. In the native range, the soil surface was mainly covered by litter (>73% vs. <38.5% in the invaded range) which consisted to a much higher degree of leaves from deciduous trees, bark and twig pieces than did the litter layer in the invaded range. In the invaded range, bryophytes dominated the soil surface (~65% vs. <24%). Furthermore, the cover of the herb layer was higher (13 and 27% in the invaded vs. <8% in the native range) and consisted often of e.g. grass tussocks and dwarf shrubs (pers. observation).

Germination (in April) and establishment (in July) of *C. claviculata* was more successful on darker sites ($r^2 = 0.09$ and

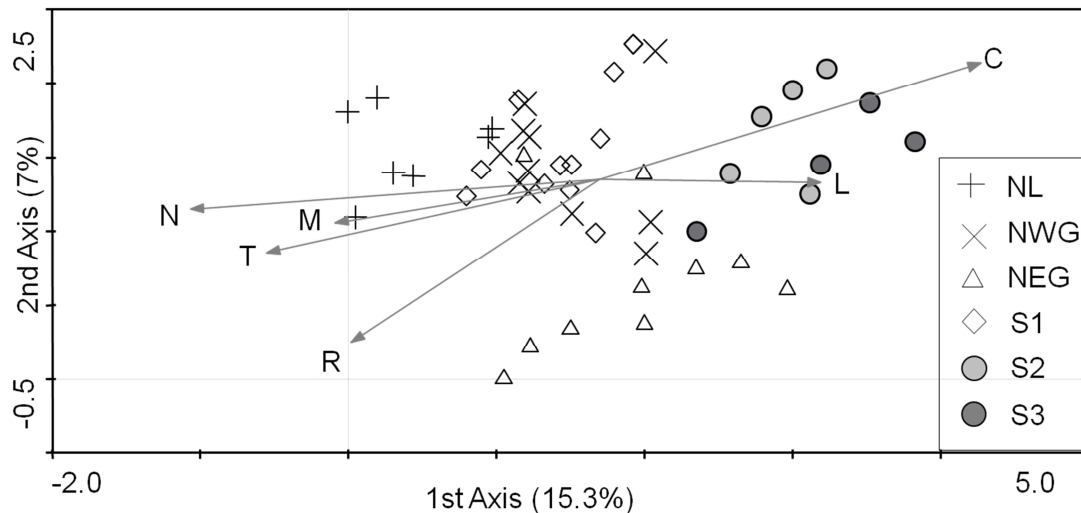


Fig. 3. Partial Detrended Correspondence Analysis (pDCA) of the vegetation in the study regions: Netherlands (NL, $n=7$), north-western Germany (NWG, $n=11$), north-eastern Germany (NEG, $n=10$), southern Sweden (S1, $n=11$), potential range in Sweden (S2, $n=5$ and S3, $n=4$). The data was adjusted for the covariable plot size. Total inertia 3.286, plot size accounted for 3.4 % of total inertia. Cumulative explained variance 29.4 %. Only the first two axes were plotted. Vectors show the correlation of Ellenberg indicator values for continentality (C), light (L), nutrients (N), soil moisture (M), reaction (R) and temperature (T) with axes scores.

Factorial common garden experiment

There was an overall germination of 23.7 % (± 1.7 % s.e.). Germination was significantly higher in shaded pots (32.13 ± 2.86 %) than in sun-exposed pots (15.35 ± 1.6 %) due to a lower risk of desiccation.

As expected, SLA was negatively correlated to sun exposition. Additionally, shaded plants showed a slender more hygrophilous appearance, were longer and had higher seed mass (Table 4). In turn, higher nitrogen supply had a positive impact on biomass, number of inflorescences and number of seeds/capsule. Capsules/inflorescence increased with sun exposure as well as with nitrogen fertilization (Table 4).

Some of the studied traits differed between the seeds of the four regions of origin such as germination, biomass, length and number of capsules/inflorescence. We found the same patterns like in the reciprocal seeding experiment; germination ranged between 41.9 ± 3.0 % in seeds from NWG and 3.9 ± 0.5 % in NEG. However, NWG and S showed in respect to germination similar behavior (also length, biomass and inflorescences). Despite these results, testing native versus invaded origin showed a higher germination of native seeds ($p < 0.001$), longer individuals ($p < 0.05$) but fewer capsules/inflorescence ($p < 0.001$, not shown).

Finally, we found significant interactions between the factors region of origin and habitat quality for the traits biomass, length, inflorescences (highly correlated to biomass: $r^2 = 0.67$, $p < 0.001$), capsules/inflorescence and seeds/capsule (not shown).

Discussion

In our study we could confirm that *C. claviculata* behaves as winter and summer annual species but with a much higher proportion of summer annual seeds. Furthermore, we detected plastic responses with respect to the factors sun exposure and nitrogen in the common garden factorial experiment. Of course, as we did not use seeds from defined single genotypes this plastic response might include a genetic component. However, population genetic analyses showed that within the studied regions the species exhibits extremely low genetic diversity (chapter 5). Therefore, we may conclude that *C. claviculata* shows moderate (regarding nitrogen) to pronounced (regarding sun) phenotypic

plasticity to the studied factors and traits. Examples of phenotypic plasticity have been observed in many

Table 4. Factorial ANOVA results of common garden experiment for all response variables. Shown are the factors habitat quality (df=3, consisting of the levels N+L+, N+L0, N0L+, N0L0) and region (df=3, consisting of the four different seed origins NL, NWG, S and NEG) and the interaction of both (“habitat quality” × “region”, df=9). F statistic (*F*), Mean of squares (*ms*), p value (*p*) and sample size (*n*), ^t = data was transformed prior analysis, ^r=ANOVA was calculated with residuals of linear regression.

	<i>n</i>	<i>MS</i>	<i>F</i>	<i>p</i>
Germination^{t,r}	192			
Habitat quality		0.792	19.481	0.000
Origin		3.014	74.135	0.000
Habitat quality × Origin		0.048	1.183	0.309
Biomass^r	160			
Habitat quality		64.35	4.841	0.003
Origin		69.46	5.225	0.002
Habitat quality × Origin		46.70	3.513	0.001
Length	171			
Habitat quality		380.65	3.854	0.011
Origin		13444.14	136.106	0.000
Habitat quality × Origin		303.30	3.071	0.002
SLA^{t,r}	164			
Habitat quality		41.912	127.720	0.000
Origin		0.522	1.591	0.194
Habitat quality × Origin		0.175	0.535	0.847
# Inflorescences^r	170			
Habitat quality		26787.58	3.583	0.015
Origin		2276.38	0.305	0.822
Habitat quality × Origin		15249.96	2.040	0.038
# Capsules	168			
Habitat quality		18.231	11.605	0.000
Origin		16.970	10.802	0.000
Habitat quality × Origin		3.170	2.017	0.041
# Seeds/capsule	168			
Habitat quality		0.4520	4.141	0.008
Origin		0.0906	0.830	0.480
Habitat quality × Origin		0.2877	2.635	0.007
Seed mass^r	152			
Habitat quality		41129.70	3.747	0.013
Origin		25755.49	2.346	0.076
Habitat quality × Origin		11211.94	1.021	0.426

widespread species (e.g. Nicotra *et al.* 2010, Loomis and Fishman 2009, Santamaría *et al.* 2003, Frenot *et al.* 1999). Widespread plants such as *Poa annua* and *Hieracium aurantiacum* (Frenot *et al.* 1999, Loomis and Fishman 2009) and many aquatic plants (Santamaría 2002) also exhibit limited genetic variation, suggesting the occurrence of general-purpose genotypes and phenotypic plasticity. These properties may have a fitness advantage in founder populations where local adaptation has not occurred yet because of a lack of genetic variation (Bossdorf *et al.* 2005) or as a strategy to respond adequately to different habitat conditions despite low genetic variation. Phenotypic plasticity may be enhanced by processes conveying intragenomic variation, like polyploidy or epigenetic variation, which may be particularly relevant for the success of species during range expansion (Prentis *et al.*, 2008, Pandit *et al.*, 2011). During early invasions plants may benefit from polyploidy if individuals with higher ploidy-level are stronger competitors or better colonizers than their diploid congeners. This is because polyploidy is accompanied by fixed heterozygosity which results from the fixing of divergent parental genomes. The enforced pairing of

homologous chromosomes prevents intergenomic recombination and benefits of heterosis are not diminished in later generations (Prentis *et al.*, 2008). In both invaded regions *C. claviculata* is present for about 30 years in numerous, sometimes huge populations (chapter 3). Thus, despite low genetic variation most of all in the northern half of its range the tetraploid *C. claviculata* may apparently buffer environmental variation at least partly through general purpose genotypes and phenotypic plasticity probably induced by intragenomic diversity and fixed heterozygosity (chapter 5).

In contrast, differences between origins in the common garden as well as in the reciprocal seeding experiment indicate genetic differentiation between the regions of origin which in turn might have evolved through adaptive processes to specific environmental conditions or genetic erosion in the new range during the colonization process (chapter 5). Additionally, we detected significant interactions

between several “origin” × “habitat quality” combinations (factorial experiment) and homesite advantage for establishment (reciprocal seeding) for both, native and, to a smaller degree, neophytic sites. Homesite advantage in fitness relevant traits and interactions between the factors region of origin and habitat quality may indicate local adaptation (Nicotra *et al.* 2010, Joshi *et al.* 2001, Potvin and Tousignant 1996). As both, native north-western Germany and non-native Sweden featured a generally higher germination, there are no clear patterns. However, in comparison with all other origins within one seeding region, those which were at their homesites germinated (only native origins) and established (native and invaded) better than the others. Jakobsson and Dinnetz (2005) suggested that if “origin” × “habitat quality” interactions do not show a clear homesite superiority there may be even so local adaptation. If individuals perform equally well or worse at their homesite than individuals of other origin at the same site, this may indicate local adaptation by relative performance. Thus, as long as the negative difference in fitness between the different seeding sites is smaller at the homesite this may be ongoing adaptation processes (Jakobsson and Dinnetz 2005).

This is stressed by the fact that since introduction a time span of only 30 to 40 years has passed and adaptations have not evolved in the invaded range (e.g. NEG) yet. Adaptation to local environment and beyond the range margin may actually be hindered due to high gene flow, genetic drift or generally low genetic variation (Kawecki and Ebert 2004). Comparable to *C. claviculata* the species *Mimulus cardinalis* and *M. lewisii* featured highest fitness in the range centre, reduced fitness at the range margin and zero fitness when transplanted beyond their present range limits (Angert and Schemske 2005). There were weak differences in performance between the populations of each species. Thus, border populations have probably not yet acquired the right mutations to exhibit high fitness and to extend the border (Angert and Schemske 2005). In fact, north-eastern German populations lacked genetic diversity more than any other region and did not exhibit any private alleles. Furthermore, within that region there was practically no differentiation between populations (chapter 5).

After all, it has been debated whether phenotypic plasticity shields genotypes from selection and thus inhibits local adaptation (Ghalambor *et al.* 2007). However, local adaptation and phenotypic plasticity are not mutually exclusive because selection pressure may also result in high phenotypic plasticity of a species and thus may also contribute to local adaptation (Ghalambor *et al.* 2007 and therein). Furthermore, the detected differences between origins, “habitat quality” × “origin interaction” or even homesite advantage may be plastic or non-plastic responses, they are not necessarily adaptive (e.g. in case of pleiotropic effects or genetic hitchhiking) and will probably not be of any value for the long term fitness/establishment of the species. Instead they may just be interpreted as indicators of ongoing evolutive processes (Davidson *et al.* 2011, Ghalambor *et al.* 2007, Kawecki and Ebert 2004).

Despite some indicatives for plasticity as well as local adaptation, we observed a fitness advantage of *C. claviculata* with respect to germination and establishment in the native range. Here, all individuals showed generally a higher fitness than in the range border of north-eastern Germany and Sweden. This is in line with other studies (e.g. Giménez-Benavides *et al.* 2007, Angert and Schemske 2005) but contradicts a study by Bossdorf *et al.* (2005) who found in a comparison of seven species (*Carduus nutans*: Woodburn and Sheppard 1996, *Hypericum perforatum*: Vilá *et al.* 2005, *Lythrum salicaria*: Edwards *et al.* 1998, Eckert *et al.* 1996, *Rhododendron ponticum*: Erfmeier and Bruehlheide 2004, *Senecio inaequidens*: Prati and Bossdorf 2004, *Silene latifolia*: Wolfe 2002, *Solidago gigantea*: Jakobs *et al.* 2004) that all except *H. perforatum* and *S. latifolia* showed evidence of higher fitness (population size, plant size and/or fecundity) in the neophytic range. However, this result may be influenced by the fact that most of the authors studied species which were considered problematic in the invaded region. Thébaud and Simberloff (2001) compared the size of all introduced species, invasive as well as inconspicuous ones, in their native and introduced range and found that there was no

general tendency for plants to be taller in the introduced range. Fitness advantage in the native range of *C. claviculata* may be related to differences in habitat quality in the study regions: Continentality, tree cover and litter layer increased gradually from The Netherlands, north-western Germany, Sweden (S1) to north-eastern Germany, whereas north-western Germany and Sweden (S1) were floristically most similar compared to all other regions. However, besides climatic differences we found that native sites featured a higher litter cover which, in turn, seems to have a positive impact on germination, establishment and length of *C. claviculata*. Presumably, under litter seedling emergence and growth is facilitated by attenuating climatic extremes such as cold or drought (e.g. Facelli and Pickett 1991, Eckstein and Donath 2005, Loydi *et al.* 2013). In contrast, germination was inhibited in sites with high cover of bryophytes or herbs. These results were also observed in greenhouse experiments (Peppler-Lisbach *et al.* unpublished). Germination success was highest if there were suitable microsites such as moderate moisture keeping leaf litter layer and if seeds had contact to the substrate (humus layer/soil). Under bryophytes, conifer and grass litter seedling emergence was inhibited probably through organic compounds or mechanical effects. Especially for germination of therophytic species availability of free microsites is important (Fenner and Thompson 2005, chapter 3). This is in line with differences between the sites within the seeding regions and stresses the importance of microsite specific conditions in the populations for germination and establishment success. Our observations correspond to the results of a reciprocal study on the three annual species *Biscutella didyma*, *Bromus fasciculatus* and *Hymenocarpus circinnatus* where local environmental conditions had larger effects than regional climate (Petrů and Tielbörger 2008). These findings may explain that despite large climatic differences between native and invaded range there are partly only small floristic differences and similar results for fitness/establishment of *C. claviculata* in Sweden and north-western Germany.

Concerning further expansion, the neophytic range is still within the physiological/ecological limit of the species. However, under the given, more sun exposed habitat conditions and more continental climate, *C. claviculata* was not able to establish beyond its current range. Thus, the species seems to be in equilibrium with its potential distribution as it has reached its natural range margin under current climatic conditions and thus, will probably not disperse much further north or east into regions with habitat conditions similar to the Swedish study sites S2 and 3. Apart from higher continentality, abiotic and biotic conditions such as lower nutrient supply, higher proportions of dwarf shrubs, bryophytes and lichens might additionally hamper germination and establishment. A negative impact of bryophytes and dwarf shrubs was observed in greenhouse experiments (chapter 3) and field studies (chapter 2). However, to draw conclusions about the potential for further expansion of *C. claviculata* and expansion rate under ongoing climate change, more detailed studies such as spatial analysis of potential habitats or range models would be necessary.

Finally, behind our results experimental difficulties may hide: Due to logistic reasons, we used mixed seed samples from the respective region for the reciprocal experiment. Thus, we could not set up the experiment in the populations where we collected the seeds originally. Becker *et al.* (2008) found in a reciprocal transplant experiment with *Hypochoeris radicata* no general differences in habitat quality among the different regions. However, there were strong effects within the seeding regions between the transplant sites and plots indicating that local environmental heterogeneity was important for the performance of the transplants and may favour evolutionary processes. Considering that we detected also microsite specific fitness differences between the sites within one region we cannot exclude that using the “real” homesite would have caused clearer results of homesite advantage. On the other hand, unlike *H. radicata*, *C. claviculata* showed much smaller genetic differentiation between the populations of one region.

Conclusions

Phenotypic plasticity and general purpose genotypes may explain range expansion and establishment success in the neophytic range despite low widespread genetic uniformity.

In contrast, there was small but detectable home site advantage to the regions of origin and a generally higher fitness in the experimental sites in the more atlantic native range. Despite this, the species has successfully established during the past decades also in the neophytic range which might be related to suitable microsites for germination. However, under current climatic conditions *C. claviculata* will most probably not expand further into more continental sites and seems to be in equilibrium with its potential range.

Due to the relatively short duration of the study we detected neither clear evidence for local adaptation to one region nor evidence for a general purpose genotype which may indicate ongoing evolutionary processes in *C. claviculata*.

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5. Range expansion of a selfing polyploid plant despite widespread genetic uniformity

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Abstract

Ongoing and previous range expansions have a strong influence on population genetic structure of plants. In turn, genetic variation in the new range may affect the population dynamics and the expansion process. The annual *Ceratocarpus claviculata* expanded its atlantic European range in recent decades towards north and east. Patterns of genetic diversity were investigated across the native range to assess current population structure and phylogeographic patterns. A test was then made as to whether genetic diversity is reduced in the neophytic range and an attempt was made to identify source regions of the expansion.

Samples were taken from 55 populations in the native and 34 populations in the neophytic range (Sweden, north-eastern Germany). Using amplified fragment length polymorphism markers an analyses was made of genetic variation and population structure (Bayesian statistical modelling) and population differentiation was quantified. Pollen/ovule ratio was analysed as a proxy for the breeding system.

Genetic diversity at population level was very low (mean $H_e=0.004$) and two multilocus genotypes dominated large parts of the new range. Population differentiation was strong ($F_{ST}=0.812$). These results and a low pollen/ovule ratio are consistent with an autogamous breeding system. Genetic variation decreased from the native to the neophytic range. Within the native range, H_e decreased towards the north-east, whereas population size increased. According to the Bayesian cluster analysis, the putative source regions of the neophytic range are situated in north-western Germany and adjacent regions.

C. claviculata shows a cline of genetic variation due to postglacial recolonization from putative Pleistocene refugia in south-western Europe. Nevertheless, the species has expanded successfully during the past 40 years to southern Sweden and north-eastern Germany where it occurs as an opportunistic neophyte. Recent expansion was mainly human-mediated by single long-distance diaspore transport and facilitated by habitat modification.

Keywords

AFLP, anthropogenic dispersal, autogamous, *Ceratocarpus claviculata*, founder effects, genetic differentiation, genetic diversity, global change, neophyte, postglacial colonization, range expansion, therophyte

Introduction

Processes of global change such as climate warming, eutrophication, human-mediated long-distance transport and habitat modification foster invasions and range expansions of plant species (Walther *et al.* 2005, Maskell *et al.* 2006, Thuiller *et al.* 2006, Wilson *et al.* 2009). Both, invasions and range expansions may impact not only the distribution of species but also the intraspecific patterns of genetic variation (Olivieri 2009, Gurevitch *et al.* 2011). In turn, genetic variation in the new range may affect or constrain the population dynamics and the expansion process (Olivieri 2009, Lachmuth *et al.* 2010).

Current patterns of genetic diversity and variation in European plant species are the results of both, past phylogeographic processes and more recent anthropogenic influences. After the climatic oscillations of the Quaternary ice ages, the previous glacial and periglacial areas were recolonized. Across their ranges, many species show a “southern richness – northern purity syndrome” characterized by a gradient of high genetic diversity of populations at low latitudes and low diversity at higher latitudes (Hewitt 2000). Populations at the rear, southern, edge were often able to persist during Quaternary oscillations through relatively small altitudinal shifts, while following matching suitable climatic conditions. Such relict populations may feature high levels of differentiation among populations and low levels of within population diversity, indicating local adaptation (Hampe and Petit 2005). In contrast, at the northern range margin, repeated range contractions have probably eliminated various genotypes. During postglacial recolonization, rapid expansion by populations from the leading edge and from a limited number of single refugial populations was accompanied by successive losses of alleles along the colonization pathway through repeated bottleneck effects and founder events (Hewitt 1999, Wilson *et al.* 2009). This pattern of “rear vs. leading edge” contrasts with a general “centre-periphery”-pattern predicting that marginal populations are genetically less diverse than those from the centre of a species distribution range (Sagarin and Gaines 2002).

Natural and anthropogenic dispersal processes have differently affected patterns of genetic diversity, which may influence later establishment success. During natural migrations from the leading edge, species expand first by short-distance dispersal according to the typical dispersal distance of their propagules via diffusion or via corridors, which link previously separated suitable areas (Hewitt 2000). Second, rare long distance dispersal events enable colonization of potentially suitable areas distant from the current range (Arrigo *et al.* 2010, Hampe 2011). Due to the rarity and stochasticity of successful long distance dispersal, populations in the new range are expected to show strong founder effects and may diverge genetically from source populations (Ibrahim *et al.* 1996). Typically, clines of genetic diversity towards the northern range edge are explained by such sampling effects during range expansion (Hewitt 1999), which in turn allow routes of invasion to be disentangled. In contrast to natural dispersal pathways, human-mediated dispersal processes tend to introduce larger numbers of propagules from more diverse sources over shorter periods of time (Simberloff 2009, Wilson *et al.* 2009). Consequently, genetic diversity in introduced populations of invasive species often has been found to be equal to that of native populations so that no pronounced genetic bottlenecks were detectable (Bossdorf *et al.* 2005). Multiple introductions from different source populations or strong propagule vectors may have compensated for losses of genetic diversity (Roman and Darling 2007, Lachmuth *et al.* 2010). In addition, admixture of different source regions in the new range may even lead to higher genetic variation and may result in higher physiological plasticity (Novak and Mack 2005, Wilson *et al.* 2009). However, in the case of anthropogenic dispersal, genetic diversity may be reduced in the invasive range because of bottlenecks during the invasion process or as a consequence of genetically impoverished source populations that served as origin for the founder individuals (Durka *et al.* 2005). Thus, propagule pressure, the number of introduction events and the source regions as well as the genetic structure within the source region are decisive factors for genetic

diversity and establishment in the target region and should be taken into consideration when studying range expansions.

Plant breeding systems have a strong influence on both, genetic diversity of populations and the population dynamics and thus may have an impact on expansion success (Hao *et al.* 2011). Self-fertilizing species with short life cycles such as many weeds are often successful and fast colonizers. If there is only a low number of diaspores introduced, self-fertilization is advantageous for fast population growth (Barrett *et al.* 2008). However, the degree of self-fertilization may vary within and among populations of a species. Because within narrow phylogenetic groups the breeding system may be indicated by pollen to ovule ratios (Cruden 1977, Michalski and Durka 2009), changes in the rate of self pollination during range expansion can be expected by changes in pollen to ovule ratios (e.g. Thomas and Murray 1981). Self-fertilizing species produce less pollen grains and thus, have a lower ratio of pollen grains to ovules per flower than related outcrossing taxa. In contrast, outcrossing plants need compatible partners and insect-pollinated species depend on pollinators. Furthermore, highly selfing species are genetically less diverse at the population level and therefore are expected to show lower evidence of genetic bottlenecks than outcrossing species (Brown and Marshall 1981). Selfing species also are less likely to suffer from inbreeding depression because deleterious alleles tend to be purged through selection (Barrett and Charlesworth 1991). Despite this, reduced genetic diversity in selfing species may also have long term negative impacts due to a reduced evolutionary potential for adaptation to changing environments (Leimu *et al.* 2006, Olivieri 2009). However, a number of successful large-scale invasions despite very low genetic variation have been reported (*Bromus tectorum*: Novak *et al.* 1991, *Reynoutria japonica*: Hollingsworth *et al.* 1998, *Rubus alceifolius*: Amsellem *et al.* 2000, *Pennisetum setaceum*: Le Roux *et al.* 2007, *Ferula loscosii*: Perez Collazos *et al.* 2009, Grimsby and Kesseli 2010, *Eichhornia crassipes*: Zhang *et al.* 2010, *Rosa rubiginosa*: Zimmermann *et al.* 2010). The success of populations lacking genetic variation might be based on either niche matching (Pérez *et al.* 2006) or on the presence of general purpose genotypes, which exhibit high phenotypic plasticity (Richards *et al.* 2006, Roman and Darling 2007). Annual selfing plants represent the “blueprint” of a successful colonizer (Baker 1967, but see Petit *et al.* 2004 and references therein). However, recent reviews (Bossdorf *et al.* 2005, Wilson *et al.* 2009) on the genetic structure of invasive plant species included only few annual selfing species and are thus biased towards perennial and outcrossing species. Therefore, in the present study we analysed genetic diversity and structure of populations of *Ceratocarpus claviculata* in its entire native and recently colonized neophytic range. The species is a selfing, annual forest herb native to western Europe. During the past 40 years, it has expanded its range further towards north-east into temperate and subcontinental regions occupying similar habitats as in the native range (chapter 2). Both, climate change and atmospheric nitrogen deposition (Lethmate *et al.* 2002) have been suggested to facilitate the expansion of *C. claviculata*. Also, anthropogenic transport of seeds as contaminants of pulpwood (Often *et al.* 2006) may have enhanced dispersal. In addition, disturbance of sites through forest machinery may create safe sites for establishment and colonization (chapter 2).

We studied population genetic patterns across the entire range of *C. claviculata* and characterized putative dispersal pathways of the rapidly expanding species. We addressed the following questions:

1. How do genetic diversity and differentiation vary across the native range?
2. Is genetic diversity reduced in the neophytic range despite successful expansion?
3. Does *C. claviculata* feature a similar degree of autogamy in populations in the native and neophytic range as reflected by pollen to ovule ratios?
4. What are the potential source regions of the expansion?

Material and Methods

Study species

The biology of *Ceratocapnos claviculata* (L.) Lidén (Papaveraceae) has been reviewed recently (chapter 3). It is a tetraploid ($2n=4x=32$) summer and winter annual with a climbing habit. The species has an eu-atlantic distribution and occurs originally in semi-shaded open oak-birch forests or substitute communities such as pine forests or on sun-exposed forest clearings. Furthermore, it is found in fringes, hedgerows or ditches along roads. In the neophytic range it occurs mostly in substitute communities as above. Its small flowers may be pollinated by insects (Apidae, Bombyliidae) but selfing due to autonomous self-pollination often occurs (Lidén 1986). The main flowering time lasts from June to September. An average individual produces about 300 black, shiny seeds of 1.3mg. Seeds have an inconspicuous, tiny aril and lack appendages for animal or wind dispersal. Thus, under natural conditions long distance seed dispersal should be rare.

Study area and sampling

We sampled 89 populations across the entire range of *C. claviculata* (Fig. 1 and Appendix S1), 34 of which were from the neophytic range in Sweden ($n=16$), eastern Germany ($n=17$) and northern France ($n=1$). The northern French population was discovered about 15 years ago (Decocq 2000) in a region, where the species had not previously been observed. All other populations ($n=55$) were situated in the native range. Populations WG 14-18 (cf. Fig. 1) represent old outposts of the native range, where the species has already been found before 1930. In the neophytic range, we visited almost all currently known localities of the species. Preliminary genotyping analyses had shown low genetic variation within populations. We therefore maximized the number of populations and limited the number of samples within populations. In each population we sampled, if possible, 4 plants randomly along a line transect, collecting young, fresh leaves for genetic analysis, resulting in a total of 342 samples. Leaves were dried and stored in silica gel at room temperature. Additionally, to determine the pollen/ovule ratio, we collected flower buds shortly before anthesis from three of the four sampled individuals and stored them in 70% ethanol. Finally, we estimated population size on a logarithmic scale (1-100 individuals=1, 101-1000=2, 1001-10 000=3, >10 000=4) by walking line transects across populations.

Breeding system

The ratio of pollen grains to ovules per flower can be indicative of the mating system within phylogenetic lineages (Cruden 1977, Michalski and Durka 2009). For characterization of the breeding system, we estimated the pollen/ovule ratio and related it to values of other Papaveraceae. For 233 flowers we suspended the total amount of pollen in 100µL water and counted pollen grains in five 2µL-aliquots under a microscope. Ovules were prepared and counted under a dissecting microscope.

Population genetic analysis

Amplified fragment length polymorphisms (AFLP, Vos *et al.* 1995) were generated following Kloss *et al.* (2011) with four primer combinations (ACT/CTA-FAM, ACA/CTA-VIC, AAG/CAC-NED, AGG/CAT-PET). For genotyping we used peak-height raw data, adjusted an individual peak height-threshold (between 80 and 800 rfu) for each locus and kept only highly reproducible loci. Across all 342 samples of *C. claviculata* this resulted in a total of 108 loci, all of which were used for data analysis and 40 of which (37.0%) were polymorphic. The error rate was tested on 29 samples that were repeated from the same DNA extract. The error rate was very low (0.25%; 12 mismatches in 4727 locus x individual pairs). The correlation between fragment size and fragment frequency was $r=-0.073$ ($P=0.451$) indicating absence of size homoplasy.

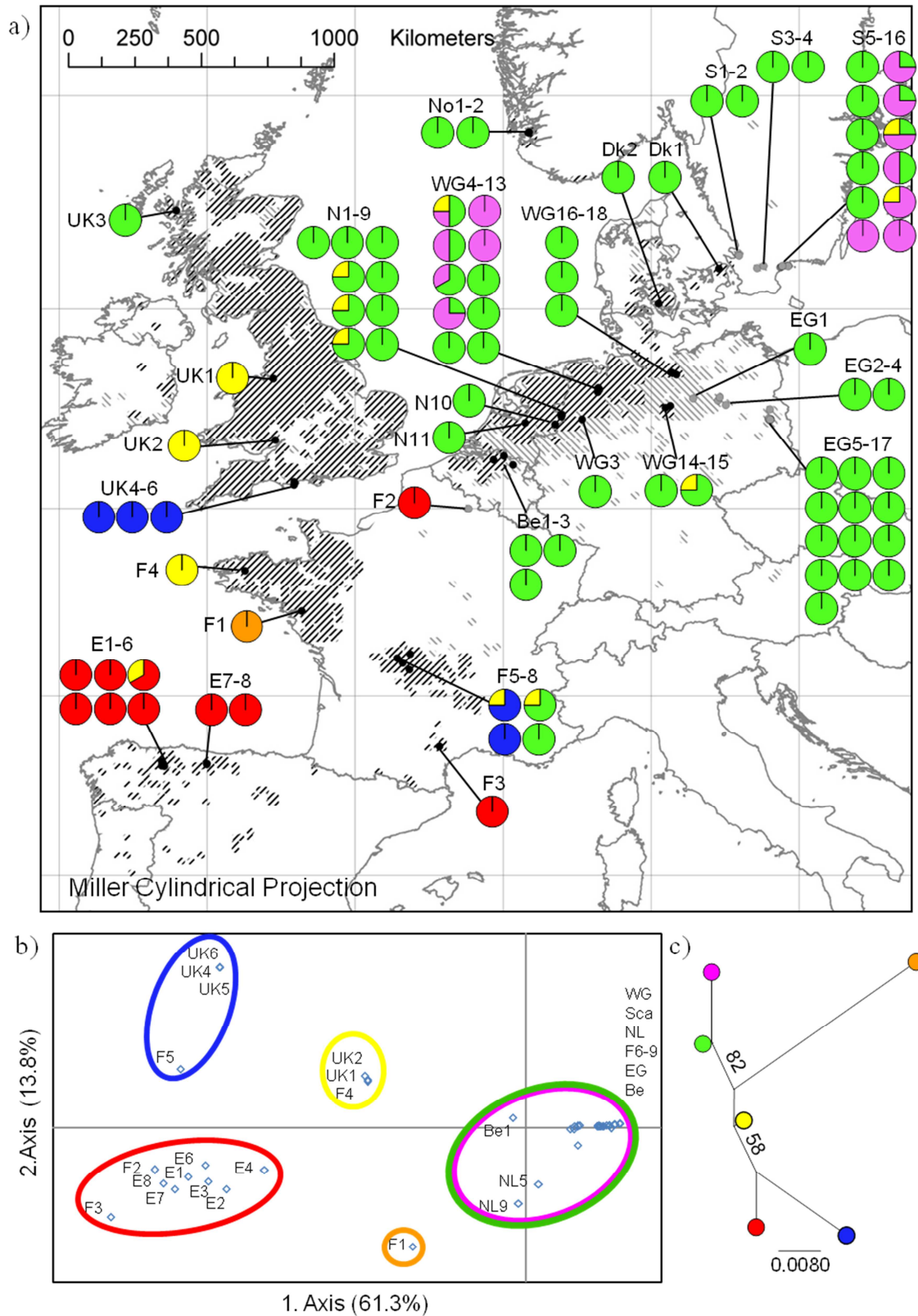


Fig. 1. Distribution of native (black hatched) and neophytic (grey hatched) range, sampling sites (cf. Appendix S1) and genetic structure of *C. claviculata*; A) BAPS cluster membership (genetic groups denoted by different colours, pie charts show the affiliation of individuals to genetic groups). B) PCoA of populations based on Nei's genetic distance. Only the first two axis are shown which accounted for 75.1% of variation (three axes explained 84.0%). Colours of the circles indicate the BAPS cluster to which the majority of individuals were affiliated. C) Neighbour-joining tree of BAPS clusters based on Nei's distance. Numbers at the branches show bootstrap support (>50%) based on 100 replicates.

Genetic variation within populations and regions was estimated as percentage polymorphic loci (*PLP*) at the 5% level, i.e. loci with allelic frequencies in the range of 0.05 to 0.95. Assuming fixed homozygosity in this highly selfing species, allele frequency was set equal to AFLP fragment frequency and expected heterozygosity (gene diversity, H_e) was calculated. The calculations were performed in AFLP-SURV v. 1.0 (Vekemans 2002) following the approach of Lynch and Milligan (1994). The number of private alleles occurring in only one population and the number of rare alleles with a frequency of $\geq 5\%$ occurring in $\leq 50\%$ of all populations was assessed manually.

Genetic population structure was analysed using a Bayesian statistical model using BAPS 5.2 (Corander *et al.* 2006). BAPS searches for genetically homogenous groups of individuals. We performed non-spatial clustering with unlinked marker data with a maximum number of clusters set to $K=15$ and without taking their population affiliation into account. Relationships among clusters were further analysed by neighbour joining of Nei's genetic distance in PHYLIP v. 3.68 (Felsenstein 2004). The robustness of each node was estimated by bootstrapping distance matrices with 100 replications in AFLP-SURV. Additionally, we conducted a Principal Coordinates Analysis (PCoA) in GeneAEx 6.41 (Peakall and Smouse 2006) to visualize the relationships among populations.

Genetic differentiation among populations was quantified with Wright's F -statistics in AFLP-SURV and differentiation among populations, native and neophytic range and among BAPS groups was quantified by analyses of molecular variance (AMOVA, Stewart and Excoffier 1996) with 1000 permutations in GeneAEx 6.41. We tested whether population structure followed an isolation by distance pattern by correlating pairwise Nei's genetic distance to geographical distance and performing a Mantel test in FSTAT v. 2.9.3.2 (Goudet 2001).

Statistical analysis

ANOVA and Tukey's HSD (honestly significance difference) test were used for unequal sample size as post-hoc test to compare genetic diversity between regions, between native and neophytic range and between BAPS groups. For the BAPS analysis, we assigned admixed populations to the prevailing group. Similarly, ANOVAs were performed to compare pollen/ovule ratios between the regions and between native and neophytic range. We used linear regression to test whether diversity could be explained by population size classes or latitude and longitude. In these analyses only populations sampled with at least three individuals were included. To meet assumptions of normality data were square root transformed before analysis whenever necessary. These calculations were performed in STATISTICA 9.1 (StatSoft Inc. 2010).

Results

Pollen-ovule ratio

C. claviculata produced a mean of 2.19 (± 0.03 s.e., $n=245$) ovules per flower and a mean of 504 (± 10 s.e., $n=245$) pollen grains per flower. The mean pollen/ovule ratio of 245 (± 7 s.e.) was ten times lower than most other, yet outcrossing, Papaveraceae species (Appendix S2), indicating an autogamous breeding system. The pollen/ovule ratio differed slightly between geographical regions (Appendix S2, ANOVA: $F_{5,239}=3.647$; $P<0.01$), but there was no significant difference between native and neophytic range (t -test: $P=0.272$).

Genotypic and genetic variation

Across all 342 individuals of *C. claviculata*, only 46 multilocus AFLP genotypes were detected. This indicates very low levels of genetic variation. In 58 out of 89 populations (65%) only a single multilocus genotype was found (Appendix S1). The most abundant multilocus genotype "A" (207 individuals, 60.5%) occurred in 61 populations and was the sole genotype in 25 populations. The

second most abundant genotype “B” (35 individuals, 10.2%) was found in 12 populations. In 11 populations a single private allele was found (Be1, WG11, E4, F2, F4, F5, UK1, UK2, EG2, EG8, S8) and two private alleles were found in two Spanish populations each. Thus, estimates of genetic diversity of *C. claviculata* at population level were very low (Table 1): mean $PLP=0.8\%$ (± 0.15 s.e., $n=89$), mean gene diversity $H_e=0.004$ (± 0.0008 s.e.) and at the species level $H_1=0.022$.

Genotypic variation was higher in the native than in the neophytic range with 39 and 10 genotypes found in the two regions, respectively. Also genetic variation was higher in the native than in the neophytic range, both at population (t -test: $P<0.01$) and overall level for gene diversity (Table 1). However, H_e also differed significantly between geographical regions and showed a decline both from south to north and from west to east (Fig. 2), essentially representing a decrease of genetic variation with distance from Spain.

The proportion of large populations (≥ 1000 individuals) tended to be higher in the neophytic range (41%) than in the native range (20%; but χ^2 -test: $P>0.05$, $n=77$) and population size was positively correlated with latitude and longitude ($r^2=0.1$, $P<0.01$; $r^2=0.07$, $P<0.05$; $n=77$). However, genetic variation was not correlated with population size ($r^2_{\text{all}}=0.03$, $P>0.05$; $n=77$).

Table 1. Values of genetic variation across populations in the native and neophytic range and in clusters identified in the BAPS analysis: Number of populations and individuals (n_p and n_i), expected heterozygosity (H_e), standard error (\pm s.e.), percentage polymorphic loci at 5% level (PLP), total number of alleles, rare alleles and private alleles and number of genotypes.

	n_p	n_i	H_e	\pm s.e.	PLP	\pm s.e.	#Alleles	#Rare Alleles	#Private Alleles	#Genotypes
Population means										
native	55	207	0.0053	0.0012	1.03	0.229	105	19	0	39
neophytic	34	135	0.0021	0.0007	0.44	0.143	89	3	0	10
overall	89	342	0.0041	0.0008	0.80	0.154	108	0	0	46
Clusters										
C1-Red		35	0.0272	0.0080	8.3		91	2	5	19
C5-Green		229	0.0017	0.0009	0		86	0	4	5
C3-Blue		16	0.0113	0.0054	4.6		87	2	2	4
C2-Yellow		22	0.0348	0.0096	6.5		95	2	7	14
C6-Pink		35	0.0005	0.0005	0		84	0	1	2
C4-Orange		4	0	0	0		81	0	0	1

Population structure and genetic differentiation

Bayesian analysis of population structure revealed six clusters with a clear geographical pattern (Fig. 1). Cluster 1 (red) comprised most of the Spanish and some French samples. Cluster 2 (yellow) consisted of populations in France, the UK and single individuals in other regions. Cluster 3 (blue) was confined to France and the UK while Cluster 4 (orange), consisting of a single genotype, occurred only in one population in France. Cluster 5 (green) included genotype “A” and was dominant in the coastal lowlands of Belgium, The Netherlands and northern Germany and Scandinavia. Cluster 6 (pink), including genotype “B”, was closely related to Cluster 5 and occurred only in north-western Germany and Sweden. The neighbour-joining tree of clusters indicated a major separation between south-western Europe (clusters 1 and 3) and north-eastern Europe (clusters 5 and 6) (Fig 1c). The clusters differed strongly with respect to genotypic and genetic variation. Clusters 1 and 2 harboured by far the largest number of genotypes and showed higher H_e while the more northern clusters 5 and 6

had the lowest H_e (Table 1). Results of the BAPS analysis were largely corroborated by the PCoA (Fig. 1b) which revealed 5 groups.

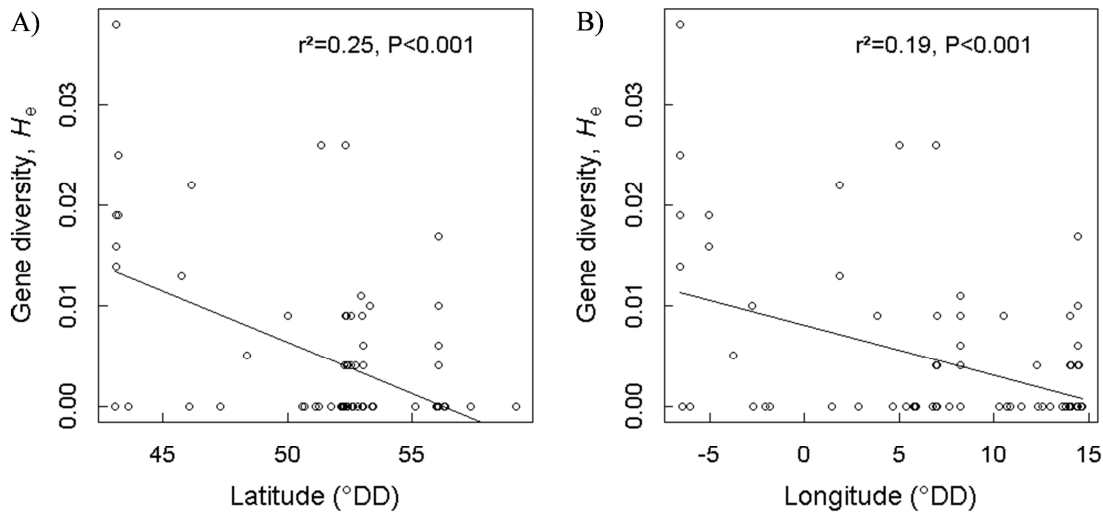


Fig. 2. Population level gene diversity H_e in relation to A) latitude and B) longitude; only populations with at least three samples are considered, $n=87$.

Wright's F -statistic indicated very strong population differentiation (overall $F_{ST}=0.812$) as expected from the very low levels of within population variation. Hierarchical AMOVA showed that differentiation between the native and neophytic range accounted for 13% of variation whereas most variation resided among populations within ranges (65%, Table 2). We found higher differentiation in the native range ($\Phi_{ST}=0.766$) than in the neophytic range ($\Phi_{ST}=0.455$) due to low levels or lack of variation in the latter. Across the entire range there was a weak pattern of isolation by distance for Nei's genetic distance ($r=0.64$, $P=0.05$, Mantel-test); this was not present when neophytic or native range were analysed separately ($P>0.225$), indicating a predominant role of genetic drift relative to gene flow.

Table 2. Summary results of analyses of molecular variance (AMOVA) of *C. claviculata*. All values are significant at $P < 0.001$.

	Overall F_{ST}	Percentage of variation		
		Among groups	Among populations	Within populations
Range				
Total range	0.760		76	24
Native range	0.766		77	23
Neophytic range	0.455		46	54
Groups				
Native vs. neophytic	0.776	13	65	22
BAPS clusters	0.849	77	7	15

Putative source regions of the neophytic range were apparent from the cluster memberships. North-western Germany was the likely source region of the Swedish invasive populations as these were the only regions that shared Cluster 6 (pink). The eastern German neophytic area was affiliated to

Cluster 5 (green) and consisted almost exclusively of genotype “A”, suggesting that the adjacent native area with the same genotype was the most likely source region. The single newly established population in northern France was part of Cluster 1 (red) suggesting southern French or Spanish populations as source region.

Discussion

Genetic diversity and structure across the native range

Overall, *C. claviculata* showed low levels or total lack of genetic variation within populations across large areas and pronounced population differentiation. This is characteristic of many selfing species (Barrett and Husband 1990, Nybom 2004) and thus was expected due to an autogamous breeding system which was also corroborated by the relatively low pollen/ovule ratio compared to other Papaveraceae.

Across the native range, we still found significant differences in genetic diversity between populations in the southern part of the range, namely France and Spain, and the northern part, namely UK, the coastal lowlands of Belgium, Netherlands and western Germany and Scandinavia. Our findings are thus in line with a general pattern of a latitudinal cline of genetic diversity from southern Europe to northern Europe (Hewitt 1999, Hampe and Petit 2005). Lower genetic diversity in the northern parts of the range may be related to postglacial recolonization processes. Range contractions and expansions during the Quaternary in the course of climatic oscillations may have eliminated genotypes in northern Europe but not those in the refugia. During recolonization of the northern European sites a loss of alleles may be the result of rapid expansion of already impoverished populations from the leading edge or from single refugial populations (Hewitt 1999). Additionally, founder effects and an autogamous breeding system may have enhanced genetic erosion (e.g. Durka 1999, Prentis *et al.* 2008). Thus, patterns of genetic variation in the native range of *C. claviculata* do not follow the central-marginal model but show a rear edge vs. leading edge pattern.

The current distribution of *C. claviculata* and models of the distribution of climatic zones during the Pleistocene suggest that the Iberian Peninsula served as a refugium (chapter 3) which is corroborated by the comparatively high levels of genetic variation found in this region. However, France also had relatively high diversity levels and harboured members of five of six genetic clusters. Thus, atlantic regions north of the Pyrenees may have represented additional refugia as has also been proposed for other species such as the oceanic orchid *Himantoglossum hircinium* (Pfeifer *et al.* 2009) and the European-wide distributed *Corylus avellana* (Palmé and Vendramin 2002).

Causes and consequences of range expansion

C. claviculata was first observed in Sweden in 1958 (Bjuv, Skåne; Hylander 1971). It has been hypothesized that seeds of this and other species may have been introduced with imported uncorticated pulpwood after heavy storms and windthrows (Oredsson 2005). Two such occasions when large amounts of timber and pulpwood (>250 000 m³) were transported from north-western Germany and The Netherlands to south Swedish saw mills and paper plants occurred in 1972 and 1976 (Oredsson 2005). Our results support this hypothesis as the introduced populations in Sweden were probably of north-western German origin. Although it is impossible to identify the precise source region for the eastern German invasive populations, they probably also originated from adjacent areas in north-western Germany. Anthropogenic dispersal probably explains the long distances (>50km). Interestingly, in northern France a single population, which reportedly was newly established, belonged to the genetic cluster found in Spain and southern France. Thus, the species seems to be strongly dispersal limited and anthropogenic long-distance seed dispersal is the principle cause for its range expansion. Like other species that are mainly distributed in forests, recent massive human

intervention such as clearcuts and transport of wood or saplings and habitat modification facilitated and accelerated expansion (Often *et al.* 2006, D'Andrea *et al.* 2009, Wilson *et al.* 2009).

Northward range expansion as a response to recent climate change has been reported in a number of plant species (Thuiller *et al.* 2006). The northern range margin of the Atlantic species *Ilex aquifolium* parallels the 0°C isoline. In recent decades this isoline has shifted northwards, as has the northern margin of *Ilex* (Walther *et al.* 2005). For *C. claviculata* which has a similar distribution to *Ilex*, the recent range expansion also has been related to recent milder winters (Lethmate *et al.* 2002). In fact, the geographical area of the climatic niche of *C. claviculata* has expanded and climatic conditions in the native and neophytic range are very similar with only slightly increased temperature differences between winter and summer as well as the proportion of summer rain towards the recently colonized range (chapter 3). Similar to other species, the current distribution is not in equilibrium with the climatic niche, which may suggest further range expansion (Magri *et al.* 2006, D'Andrea *et al.* 2009). However, both ecologically suitable microsites for germination and human-induced dispersal appear to be prerequisites for the expansion and establishment of the annual *C. claviculata*.

The pollen/ovule ratios were similar in the old and new range, suggesting maintenance of the autogamous breeding system. However, in the new range we observed lower genetic diversity than in the old range and widespread genetic uniformity. In general, genetic impoverishment may be due to founder events and genetic bottlenecks during the invasion process (Barrett and Kohn 1991, Edwards *et al.* 2006, Okada *et al.* 2009). However, it is more likely that for *C. claviculata* pre-existing low levels of genetic diversity in the source populations are responsible. The occurrence of two BAPS cluster groups in the Swedish neophytic range suggests either a single introduction from a source population containing both clusters, or multiple introductions from several source populations from The Netherlands, Belgium or north-western Germany. The new eastern German populations lacked both genetic diversity within and differentiation between populations, suggesting a single introduction and further dispersal within eastern Germany.

Low genetic variability may limit colonization of new habitats due to lack of evolutionary potential under variable or novel environmental conditions (Stebbins 1957). However, *C. claviculata* is a successful colonizer despite very low levels or lack of genetic diversity. In fact, we observed an increase of population size from south-west to north-east of the range, possibly indicating increased fitness. This paradox may be interpreted against the background of phenotypic plasticity. Despite very low levels of genetic variation, *C. claviculata* shows highly plastic responses, e.g. of leaf morphology, to changing light conditions, enabling the colonization of various habitats (chapters 2 and 3). Phenotypic plasticity in turn may be enhanced by processes conveying intragenomic variation, such as polyploidy or epigenetic variation, which may be particularly relevant for the success of species during range expansion (Prentis *et al.* 2008, Pandit *et al.* 2011). Thus, in the polyploid *C. claviculata* despite the lack of genotypic variability, intragenomic diversity and fixed heterozygosity may account for phenotypic plasticity.

The role of phenotypic plasticity for the success of colonizing species has been classified as either a “Jack-of-all-trades” strategy (Richards *et al.* 2006) of a robust general-purpose-genotype that maintains high fitness also in unfavourable environments or an opportunistic “Master-of-some-situation” strategy, in which the colonizer is better able to increase fitness than resident species under particularly favourable conditions (Baker 1967, Richards *et al.* 2006). *C. claviculata* is able to colonize a wide range of habitats on non-calcareous soils such as open forests, fringes, hedgerows, ditches and disturbed bogs. However, data both from common garden experiments and field sites suggest that *C. claviculata* attains higher fitness (higher individual biomass) under particular conditions (chapters 2 and 3). For example, all these habitats are semi-shaded and have humic soils of intermediate moisture and some open sites in the herb layer. The latter conditions facilitate seedling

establishment, which is the most critical stage of annual species (chapter 3). Thus, the species may be assigned as a “Master-of-some-situation”-type, similar to other successful colonizers (Richards *et al.* 2006).

Finally, two methodological shortcomings should be considered related to the partitioning of genetic variation and that we sampled a large number of populations across the entire range of the species with only moderate sample sizes per population. First, the finding of private alleles in Sweden and eastern Germany might indicate that new mutations have occurred in recent decades or that we missed to detect the respective alleles in the native range. Secondly, the extremely low level of variation present in the species precluded a more stringent identification of both the genetic relationships and the demographic and historical processes in the native and neophytic range. Many of the multilocus genotypes differed only by the presence or absence of a few AFLP bands, making it impossible to assess their phylogenetic relationships. The low sample size per population may have limited the detection of shared AFLP bands and thus the amount of gene flow among populations, but this was partly compensated for by a high number of populations. Still, in certain regions, such as France, which harboured 5 of the 6 clusters, a denser sampling could advance our understanding of the history of *C. claviculata*.

Conclusions

C. claviculata is an example of a successful autogamous, therophytic neophyte with extremely low genetic diversity. Our analyses suggest Pleistocene refugia in south-western Europe (Spain, France). During the process of postglacial recolonization, the species lost a large part of its genetic variability, which is illustrated by a north-eastward decline of genetic diversity and thus increasing distance from Spain. Our data corroborate the proposed colonization of southern Sweden from sites in The Netherlands and north-western Germany, which was mediated through anthropogenic dispersal. Low genetic diversity in the newly colonized range is related to genetically impoverished source populations rather than to founder effects. Polyploidy may alleviate the low level of genetic diversity, which does not compromise range expansion. We suggest that a combination of increased opportunities for long-distance dispersal through anthropogenic activities, an extension of the climatic niche owing to recent climate change and suitable habitat quality through land-use changes may contribute to range expansion of many species.

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Appendix

S1. Regions, population codes (Population), number of samples (Samples), locations of sampled populations in WGS84 geo-coordinates in decimal degree east (E) and north (N), invasion status (Status), population size class (Size: 1=1-100 individuals, 2=101-1000, 3=1001-10 000, 4=>10 000), percentage polymorphic markers (*PLP*), gene diversity (H_e) and number of multilocus genotypes (#Genotypes).

Region	Population	Samples	E	N	Status	Size	<i>PLP</i>	H_e	#Genotypes
Coastal Lowlands									
Belgium	Be1	4	5.0197	51.3409	native	n.a.	5.6	0.026	2
	Be2	4	4.6729	51.2433	native	n.a.	0	0	1
	Be3	4	5.3332	51.1119	native	n.a.	0	0	1
Netherlands	NL1	4	6.9618	52.3301	native	1	5.6	0.026	2
	NL2	3	6.9645	52.3355	native	1	0	0	1
	NL3	4	6.9698	52.3873	native	1	0.9	0.004	2
	NL4	4	6.9475	52.3845	native	1	0	0	1
	NL5	4	6.9607	52.3794	native	2	0.9	0.004	2
	NL6	4	6.9893	52.3858	native	2	0.9	0.004	2
	NL7	4	6.9893	52.3766	native	2	1.9	0.009	2
	NL8	3	6.9656	52.3792	native	1	0	0	1
	NL9	3	6.9574	52.3758	native	1	0	0	1
	NL10	4	6.7501	52.1392	native	2	0	0	1
	NL11	4	5.7461	52.1739	native	3	0	0	1
W Germany	WG3	4	7.6650	52.2636	native	4	0	0	1
	WG4	4	8.1934	53.0305	native	2	0.9	0.004	2
	WG5	3	8.1942	52.9433	native	2	1.9	0.011	2
	WG6	4	8.2025	53.0363	native	2	0	0	1
	WG7	4	8.2099	53.0375	native	1	0.9	0.006	2
	WG8	4	8.2178	53.046	native	1	0	0	1
	WG9	4	8.2197	53.001	native	3	0	0	1
	WG10	4	8.2224	53.0051	native	2	0	0	1
	WG11	4	8.2347	52.993	native	2	1.9	0.009	3
	WG12	4	8.2401	53.0012	native	2	0	0	1
	WG13	4	8.2501	53.0079	native	2	0	0	1
	WG14	4	10.4793	52.554	native	n.a.	1.9	0.009	2
	WG15	4	10.647	52.5986	native	n.a.	0	0	1
	WG16	4	10.663	53.4325	native	2	0	0	1
	WG17	4	10.7022	53.4262	native	3	0	0	1
	WG18	4	10.8498	53.3739	native	3	0	0	1
E Germany									
	EG1	4	11.4111	52.7855	invaded	3	0	0	1
	EG2	4	12.2485	52.733	invaded	3	0.9	0.004	2
	EG3	4	12.3138	52.8153	invaded	2	0	0	1
	EG4	4	12.5165	52.6222	invaded	n.a.	0	0	1
	EG5	4	14.0095	52.5145	invaded	4	0.9	0.004	2
	EG6	4	13.9697	52.2817	invaded	3	0	0	1
	EG7	4	13.9608	52.2723	invaded	3	0	0	1
	EG8	4	13.9757	52.2953	invaded	2	1.9	0.009	3

Region	Population	Samples	E	N	Status	PLP		H_e	#Genotypes
France	EG9	4	14.0159	52.2634	invaded	2	0	0	1
	EG10	4	13.9709	52.2645	invaded	2	0	0	1
	EG11	4	14.0066	52.2808	invaded	3	0	0	1
	EG12	4	13.9768	52.2638	invaded	3	0	0	1
	EG13	4	14.034	52.2723	invaded	1	0.9	0.004	2
	EG14	4	13.9948	52.2422	invaded	1	0	0	1
	EG15	4	14.0301	52.2523	invaded	1	0	0	1
	EG16	4	14.0372	52.2678	invaded	1	0	0	1
	EG17	4	14.036	52.2601	invaded	1	0	0	1
	F1	4	-1.8073	47.2947	native	3	0	0	1
	F2	4	3.8309	49.9976	invaded	2	1.9	0.009	3
	F3	4	2.8442	43.6	native	1	0	0	1
	F4	3	-3.7218	48.3602	native	1	0.9	0.005	2
	F5	4	1.8604	46.143	native	n.a.	3.7	0.022	3
	F6	1	1.6	45.9	native	n.a.	0	0	1
	F7	3	1.4187	46.0295	native	n.a.	0	0	1
	F8	4	1.8302	45.7194	native	n.a.	2.8	0.013	2
Scandinavia									
Denmark	Dk1	4	12.2857	55.9756	native	2	0	0	1
	Dk2	4	10.2457	55.1274	native	3	0	0	1
Norway	No1	4	5.8728	59.1648	native	n.a.	0	0	1
	No2	4	5.845	59.153	native	n.a.	0	0	1
Sweden	S1	4	12.9343	56.3002	invaded	1	0	0	1
	S2	4	12.9631	56.2822	invaded	1	0	0	1
	S3	4	13.5764	55.9863	invaded	2	0	0	1
	S4	4	13.796	56.0358	invaded	1	0	0	1
	S5	4	14.3619	56.0041	invaded	3	0	0	1
	S6	4	14.3857	56.0115	invaded	2	0	0	1
	S7	4	14.4001	56.0257	invaded	4	0.9	0.006	2
	S8	4	14.4188	56.0324	invaded	3	3.7	0.017	2
	S9	4	14.4307	56.0371	invaded	2	1.9	0.010	3
	S10	4	14.4381	56.0345	invaded	4	0.9	0.004	2
	S11	4	14.4518	56.0361	invaded	4	0.9	0.004	2
	S12	3	14.5922	56.0429	invaded	1	0	0	1
	S13	4	14.6050	56.0502	invaded	2	0	0	1
	S14	4	14.6162	56.0446	invaded	3	0	0	1
	S15	4	14.6221	56.0464	invaded	1	0	0	1
	S16	4	14.6255	56.0342	invaded	2	0	0	1
Spain									
E1	4	-6.54037	43.1949	native	1	4.6	0.025	4	
E2	4	-6.54099	43.1955	native	2	3.7	0.019	3	
E3	4	-6.52793	43.1185	native	2	2.8	0.014	4	
E4	3	-6.53376	43.0969	native	1	6.5	0.038	3	
E5	1	-6.53927	43.0677	native	1	0	0	1	
E6	4	-6.45162	43.0579	native	1	0	0	1	
E7	4	-5.00835	43.1235	native	1	3.7	0.019	4	

Region	Population	Samples	E	N	Status	<i>PLP</i>		H_e	#Genotypes
United Kingdom	E8	4	-5.01647	43.1094	native	1	2.8	0.016	3
	UK1	4	-2.76947	53.2725	native	3	1.9	0.010	3
	UK2	4	-2.70313	51.7446	native	3	0	0	1
	UK3	4	-6.04550	57.3529	native	2	0	0	1
	UK4	4	-2.04167	50.6583	native	2	0	0	1
	UK5	4	-2.07333	50.6017	native	2	0	0	1
	UK6	4	-2.06081	50.6817	native	2	0	0	1

S2. Number of samples (N), mean and standard error (\pm s.e.) of ovules and pollen per flower and pollen/ovule ratio in *C. claviculata* in different regions. Letters indicate significant differences according to HSD test, ($P < 0.05$). Below: pollen/ovule ratio in related Fumariaceae species.

	N	Ovules	Pollen	Pollen:Ovule Ratio
<i>Ceratocapnos claviculata</i>				
Spain	9	2.11 (0.20)	440 ^{ab} (33)	233 ^{ab} (37)
France	38	2.08 (0.06)	537 ^b (17)	265 ^a (11)
UK	16	2.06 (0.06)	479 ^{ab} (44)	234 ^{ab} (22)
Coastal Lowlands	80	2.28 (0.05)	513 ^{ab} (17)	237 ^{ab} (11)
E Germany	28	2.25 (0.12)	414 ^a (46)	195 ^b (25)
Scandinavia	74	2.16 (0.06)	525 ^{ab} (14)	265 ^a (15)
<i>native</i>	165	2.21 (0.04)	517 (11)	249 (8)
<i>invasive</i>	80	2.15 (0.06)	477 (20)	237 (13)
<i>Ceratocapnos claviculata</i>	245	2.19 (0.03)	504 (10)	245 (7)
<i>Corydalis orthoceras</i> ^{1,7}				1717-4169
<i>Corydalis lineariloba</i> ^{1,7}				2152-3720
<i>Corydalis fumariifolia</i> ^{2,7}				3000
<i>Corydalis fumariifolia</i> ^{3,7}				1400
<i>Corydalis cava</i> ^{4,7}				3244
<i>Hypecoum procumbens</i> ssp. <i>fragrantissimum</i> ^{5,7}				4934
<i>Sarcocapnos pulcherrima</i> ^{6,8}				2780
<i>Hypecoum procumbens</i> ssp. <i>procumbens</i> ^{5,8}				465-1293

¹Fukuhara (2000), ²Sunaga (1988), ³Ohara and Higashi (1994), ⁴Christ *et al.* (2001), ⁵Dahl (1989), ⁶Salinas and Suarez (2003), ⁷self-incompatible, ⁸selfing

Summary

The eu-oceanic woodland herb *Ceratocarpus claviculata* has been expanding north- and eastwards into north temperate and subcontinental regions during the past decades. The rapid range expansion of this therophyte may be an example of a species that strongly benefits from global change. The aims of this dissertation were thus to study filters and processes which are related to the expansion success and the persistence of colonizing plant populations in native and neophytic range sites. To this end, the project focused on the following objectives: to study the biology, habitat requirements and the effects of ecological filters for colonization of *C. claviculata* across the entire range, to test for phenotypic plasticity and local adaptation in native and neophytic populations, to analyze population genetic patterns (diversity, founder effects) in the native and neophytic range and to characterize possible dispersal pathways.

In the first study I analyzed the community composition of vegetation plot data containing *C. claviculata* using ordination techniques (CCA, DCA) on three different scales: across the entire range, between the native and invaded range and during the past decades within the native range. *C. claviculata* is characterized by a broad macroclimatic amplitude. Relevés in the neophytic range showed more indicators of anthropogenic disturbance than native sites and abundance of *C. claviculata* was positively linked to silvicultural intensity. Newer relevés from after 1990 showed, e.g., higher Ellenberg nutrient indicator values, lower species diversity and higher proportions of neophytic and hemerobic species than old relevés from before 1970.

In the second study I compiled all available data and reviewed taxonomy, morphology, distribution, habitat requirements, life cycle and biology of the species.

In the third study I carried out a reciprocal seeding experiment in the native and in the neophytic range, and a common garden experiment in order to test for the presence and effects of local adaptation and phenotypic plasticity. Additionally, we tested whether the species is able to establish beyond the current range. *C. claviculata* showed phenotypic plasticity towards the factors nitrogen availability and sun exposure. However, differences between plants of different seed origins and homesite advantage in some fitness-related plant traits indicated also an ongoing local adaptation to environmental conditions. Probably due to climatic reasons, native seeding regions featured higher establishment than neophytic regions. Future expansion into more continental region seems improbable as the species did not establish successfully beyond the current range.

In the last study I investigated patterns of genetic diversity across the native range to assess the current population structure and phylogeographic patterns. I tested, whether genetic diversity is reduced in the neophytic range and tried to identify source regions of the expansion. *C. claviculata* shows generally a very low genetic diversity (mean $H_e=0.004$) and strong population differentiation ($F_{ST}=0.812$). Genetic variation decreased within the native range towards the north-east, possibly due to the postglacial recolonization from putative Pleistocene refugia in south-western Europe. Variation decreased also from the native to the neophytic range. Bayesian cluster analysis suggested that putative source regions of the neophytic range are situated in north-western Germany and adjacent regions.

Beyond potential positive effects of soil eutrophication and mild winters, direct anthropogenic impact seems to be the most important filter which determines invasibility of plant communities with *C. claviculata*. Anthropogenic impact may directly facilitate seed dispersal and provides sites and resource conditions suitable for germination and establishment of the short-lived species. Thus, despite very low genetic variation *C. claviculata* expanded successfully its range and occurs as opportunistic neophyte. This may be also enhanced through general purpose genotypes and phenotypic plasticity. However, in my field experiments a higher establishment success was found in the native range, which

may indicate more favorable environmental conditions such as nutrient availability, soil moisture, climate and floristic composition.

Zusammenfassung

Während der letzten vier Jahrzehnte hat sich die eu-ozeanische Waldart *Ceratocarpus claviculata* nord- und ostwärts in nördlich-temperate und subkontinentale Regionen ausgebreitet. Aufgrund seiner schnellen Arealerweiterung ist dieser Therophyt beispielhaft für viele andere Arten, die vom globalen Wandel profitieren. Die Absicht dieser Dissertation ist, Filter und Prozesse zu untersuchen, die mit dem Ausbreitungserfolg und der Persistenz kolonisierender Pflanzenpopulationen im ursprünglichen und neuen Areal im Zusammenhang stehen. Zu diesem Zweck konzentrierte sich das Projekt auf folgende Ziele: Biologie, Habitatansprüche und die Folgen von ökologischen Filtern zu untersuchen, die für die Kolonisierung von *C. claviculata* im gesamten Areal wichtig sind; die Art auf phänotypische Plastizität und lokale Adaptation im ursprünglichen und neophytischen Areal zu testen; populationsgenetische Muster (genetische Diversität, Gründereffekte) im ursprünglichen und neophytischen Areal zu analysieren und mögliche Ausbreitungswege zu rekonstruieren.

In der ersten Studie habe ich alle verfügbaren Daten zu Taxonomie, Morphologie, Verbreitung, Habitatansprüche, Lebenszyklus und Biologie der Art zusammengetragen.

In der zweiten Studie habe ich die Zusammensetzung von Pflanzengesellschaften und die Deckung von *C. claviculata* mit Hilfe von Ordinationsmethoden (CCA, DCA) auf drei verschiedenen Skalen untersucht: (i) im gesamten Areal, (ii) im ursprünglichen und neophytischen Areal und (iii) während der letzten Jahrzehnte innerhalb des ursprünglichen Areals. Das Vorkommen von *C. claviculata* ist durch eine große makroklimatische Amplitude charakterisiert. Vegetationsaufnahmen im neophytischen Areal wiesen mehr Indikatoren für anthropogen verursachte Störungen auf als Aufnahmen im alten Areal und die Abundanz von *C. claviculata* stand in einem positiven Zusammenhang mit forstwirtschaftlicher Intensität. Neue Vegetationsaufnahmen, die nach 1990 entstanden sind, zeigten höhere Ellenberg Zeigerwerte für Nährstoffe, eine geringere Diversität von Pflanzenarten und einen höheren Anteil neophytischer und hemerober Arten als alte Aufnahmen, die von vor 1970 stammten.

In der dritten Studie führte ich im ursprünglichen und neophytischen Areal ein reziprokes Aussaatexperiment durch und ein Experiment auf den Freiflächen des Versuchsfeldes, um das Auftreten und die Folgen von lokaler Adaptation und phänotypischer Plastizität zu testen. Darüber hinaus habe ich die Etablierung der Art außerhalb ihrer gegenwärtigen Verbreitungsgrenze untersucht. *C. claviculata* weist eine phänotypische Plastizität gegenüber den Faktoren Stickstoffverfügbarkeit und Sonneneinstrahlung auf. Allerdings gab es auch Hinweise lokaler Adaptation gegenüber vorherrschenden Umweltbedingungen, da ich Unterschiede zwischen den Pflanzen unterschiedlicher Samenherkünfte und Heimvorteile bezüglich einiger fitnessrelevanter Pflanzeigenschaften feststellen konnte. Bei der Aussaat in nativen Regionen kam es vermutlich klimabedingt zu einer höheren Etablierung als in neophytischen Regionen. Eine Expansion in Gegenden, die noch kontinentaler sind, erscheint unwahrscheinlich, da sich die Art nicht an den Versuchsflächen jenseits ihres gegenwärtigen Verbreitungsgebietes etablieren konnte.

In der letzten Studie untersuchte ich die genetische Diversität von *C. claviculata* im gesamten ursprünglichen Areal, um die gegenwärtige Populationsstruktur und phylogeographische Muster beurteilen zu können. Ich testete, ob die genetische Diversität im neophytischen Areal reduziert ist und versuchte Ursprungsregionen für die Verbreitung der Art zu identifizieren. *C. claviculata* weist allgemein eine sehr geringe genetische Diversität auf (durchschnittlicher $H_e=0.004$) und eine starke Populationsdifferenzierung ($F_{ST}=0.812$). Die genetische Variation nahm innerhalb des nativen Areals gegen Nordosten ab, was sich durch postglaziale Rekolonisationsprozesse von möglichen pleistozänen Refugien in Südwest Europa erklären lässt. Ebenso nahm die Variation vom nativen zum

neophytischen Areal ab. Bayesische Cluster Analysen lassen darauf schließen, dass die neophytische Verbreitung ihren Ursprung in Nordwest Deutschland und Umgebung hat.

Neben möglichen positiven Auswirkungen der Bodeneutrophierung und milder Winter, scheinen anthropogene Einflüsse zu den wichtigsten Filtern zu gehören, die die Invasibilität von Pflanzengesellschaften durch *C. claviculata* bestimmen. Anthropogene Einflüsse können direkt die Samenausbreitung erleichtern und für die kurzlebige Art geeignete Keimungs- und Etablierungsbedingungen hinsichtlich Wuchsorten und Ressourcen schaffen. Dadurch konnte *C. claviculata* trotz geringer genetischer Diversität erfolgreich sein Areal erweitern und als opportunistischer Neophyt vorkommen. Dies wird möglicherweise auch durch universale Genotypen und phänotypische Plastizität verstärkt. Dennoch zeigte sich in meinen Feldversuchen, dass der Etablierungserfolg im ursprünglichen Areal höher ist, was sich auf günstigere Umweltbedingungen wie Nährstoffverfügbarkeit, Bodenfeuchte, Klima und Florazusammensetzung zurückführen lassen kann.

List of publications

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- Voss N, Durka W, Eckstein RL. Homesite advantage and phenotypic plasticity in a range expanding forest therophyte. (unpublished manuscript).

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Versicherung

Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit Hilfen angefertigt, die ich in der Dissertation angegeben habe.

Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.

Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

(Nicole Voß)

Ort, Datum