

Justus-Liebig-Universität Gießen

Master of Science Agrobiotechnology



**Sensory Mechanisms of Host Recognition and the  
Action of Repellents in the Bed Bug *Cimex  
lectularius* and the Mosquito *Aedes aegypti***

Master's thesis

from

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## **I Abstract**

Mosquitos and bed bugs use blood of humans and other species as a source of nutrition. When searching for hosts, both insects express a host-seeking behavior in order to find the source. So far, literature on host recognition supports evidence that bed bugs and mosquitos are able to sense warmth of the host due to convection. Furthermore, temperature-sensitive sensory structures responding to warm air (convection) have been found on the two insects' antennae. However, heat is generally transferred in three ways: convection (warm currents), conduction (heat transfer through contact) and radiation (transfer of energy by electromagnetic waves). We have been able to obtain evidence that mosquitos have a directional infrared sense used in host-seeking. The results show that mosquitos are strongly attracted by a warm, IR-emitting source, whereas bed bug did not appear to use IR-sensing for host-seeking. Furthermore, three repellents (DEET, cinnamaldehyde and citronellal) were tested and found to disrupt infrared-guided thermotaxis in mosquitos at low spatial concentrations in air.

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# 1. Introduction

## 1.1 Introduction to Ectoparasites

Ectoparasites are organisms that live on or within the dermis of a host animal. Ectoparasites obtain vital nutrients at the expense of the host, which results in painful wounds and skin irritations and can become contaminated, leading to secondary bacterial infections. Arthropod ectoparasites are also capable of vectoring microbial agents that can cause diseases to susceptible animal hosts. A prominent example is *Aedes aegypti* as a pathogen vector of the yellow-fever virus (section 1.3.2.1. - yellow-fever) (Lehmann 1993). Ectoparasites are also of economic importance with, for example, livestock infestations, negatively impact animal fitness and subsequently decreasing milk, meat, and wool production (Lehmann 1993). Ectoparasites have a significant impact on, e.g., livestock profitability, animal health and vector microbial agents that cause diseases in both humans and animals. Therefore, the demand for effective control agents has increased (Taylor 2001) to prevent and diminish the negative impact of ectoparasites. Examples for effective control agents are, for example, repellents as DEET (Klun, Khiridian et al. 2006) and Citronella oil (Kim, Kang et al. 2005). Repellents can disrupt the host-seeking behavior and decrease, for example, the transfer of diseases (Kim, Kang et al. 2005). However, host-seeking is an important step in the life cycle of ectoparasites. For example, bed bugs and mosquitos need to locate a host to feed on blood to produce eggs. (Suchy and Lewis 2011). We have discovered a new sense involved in host-seeking, therefore we investigated the effects of repellents on that sense.

This study focused on two ectoparasites, the yellow-fever mosquito (*Aedes aegypti*) and the bed bug (*Cimex lectularius*).

## 1.2 Bed Bugs (*Cimex lectularius*)

The bed bug *Cimex lectularius* is a worldwide human insect pest found in the order Hemiptera and the family Cimicidae (Harlan 2006). Many countries in North America, Europe, Australia and some parts of Africa have reported a resurgence of bed bug infestations (Harlan 2006). After World War II, bed bugs were nearly eradicated in industrialized countries due to the heavy use of synthetic insecticides. One reason for the recurrence of the ectoparasites is globalization with, for example, immensely increased international travelling (Potter 2012). Another more important reason for the recurrence is the resistance to pest control (Myamba, Maxwell et al. 2002). The United States of America, for instance, have had experienced a dramatic comeback

of bed bugs in homes, apartments, hotels, health care facilities, dormitories, schools and public transportation (Potter 2012). In 2010, the US spent approximately 250 million dollars for bed bug management across the country (Bowers 2010). In 2004, a dramatic rise of bed bugs in Australia was reported (Doggett, Geary et al. 2004).

### 1.2.1 Biology

The bed bug *C. lectularius* is an obligatory blood feeder that requires blood meals for growth, development and reproduction. Both sexes of *C. lectularius* feed blood (Reinhardt and Siva-Jothy 2007). In order to find the host, the bed bugs need the antennae to sense the host. In general, *C. lectularius* prefer human hosts but will also seek out other warm-blooded animals such as dogs, cats, birds and rodents (Potter 2012). For the blood meal uptake, bed bugs penetrate the skin with their mouthparts for 3 to 10 minutes. After blood feeding, bed bugs immediately hide and digest the blood (Potter 2012). With one blood meal, bed bugs can survive from 3 to 15 days up to 1 year depending on the life cycle stage (Delaunay, Blanc et al. 2011). In order to reach maturity, bed bugs need to molt five times (Fig. 1) and require a blood meal for each molt (Potter 2012). Therefore, the bed bug life cycle consists of three stages: the egg stage, nymph which has 5 instars and the adult stage (Fig. 1). Mated females will lay 5 to 15 eggs per day, with an associated hatching time of 7 to 15 days. One female adult bed bug can produce 200 to 500 eggs over one life span (Harlan 2006). The egg to egg life cycle spans between 40 to 70 days and the adult life time ranges between 6 and 24 month (Delaunay, Blanc et al. 2011). Bed bugs are nocturnal (night active) and typically feed at night. Depending on the feeding status, bed bugs are also able to feed during the day on, for example, night-shift workers. After feeding, the 6-7 mm long, oval shaped bed bugs (Harlan 2006) congregate in hidden spots, without having a nest. The location is usually close “within crawling distance” to the feeding source (Potter 2012).

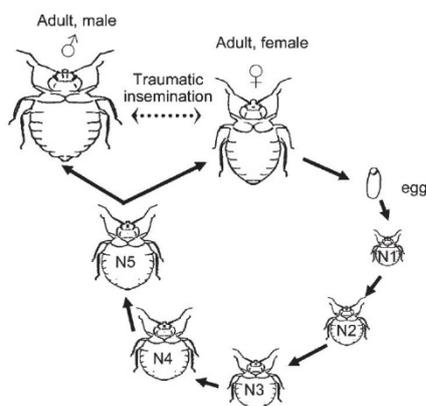


Figure 1

#### **Bed bugs life cycle**

This graph shows the three stages of the *C. lectularius* bed bugs life cycle. The cycle starts with traumatic insemination mating behavior which results in eggs. The eggs will be considered as the first stage and the five nymph stages (N1-N5) as the second stage. Every stage within the nymphs takes between 3 to 7 days and requires a blood meal. In stage three the bed bugs reach the fertile age at which the life cycle closes with another traumatic insemination mating.

### 1.2.2 Transmitted Diseases

In general, bed bugs are suspected of transmitting agents that cause infectious diseases (Delaunay, Blanc et al. 2011). However, the study of Delaunay described 45 pathogens that can potentially be transmitted via bed bugs (Delaunay, Blanc et al. 2011). The best-studied organism that can cause infectious diseases are: *Coxiella burnetii*, *Aspergillus spp.*, *Trypanosoma cruzi*, *Hepatitis B virus* (HBV) and the *human immunodeficiency virus* (HIV). For example, *C. burnetii* spores, the causative agent of Q fever, were still readily detectable in bedbugs 250 days after transfection. Bed bugs have also been associated with the translocation and spread of the mold spore *Aspergillus spp. T. cruzi* the causative agent of Chagas' disease has also been identified in bed bugs. Furthermore, Hepatitis B virus (HBV) is the best candidate for transmission by bed bugs (Delaunay, Blanc et al. 2011). However, these examples point out potential pathogens that can be transmitted by blood-feeding bed bugs. Host-seeking behavior is important for locating the host in order to feed. The main sensory organs for host location are the antennae (Reinhardt and Siva-Jothy 2007). The role of the antennae and potentially involved sensory structures in bed bugs were further investigated in this thesis as we discovered a new sense involved in host-seeking.

## **1.3 Mosquitos (*Aedes aegypti*)**

The yellow-fever mosquito *Aedes aegypti* is a tropical mosquito that can be found in parts of Europe, North America and Australia. Before World War II, *A. aegypti* appeared in the Mediterranean region but quickly disappeared during the war because of the use of insecticides (Jansen and Beebe 2010). Similar to bed bugs, *A. aegypti* mosquitos need human blood in order to produce eggs. The yellow-fever mosquito is a daytime biting mosquito, unlike many other species, which are typically active at dusk and dawn (Jansen and Beebe 2010). *A. aegypti* can transmit pathogens that cause yellow-fever, dengue-fever and zika virus (see section Transmitted Diseases).

### 1.3.1 Biology

In order to understand the immense impact of the yellow-fever mosquito as a vector and the associated host-seeking behavior, it is important to take a look at the biology. *A. aegypti* is the best characterized species within the Dipteran family Culicinae. The yellow fever mosquito life cycle consists of a complete metamorphosis with egg, larva, pupa and adult stages. However, adult female *A. aegypti* mosquitoes feed on blood, whereas males rely on nectar as a source of nutrition (Zettel and Kaufman 2012). In order to develop the eggs, *A. aegypti* mosquitoes need to feed several times on blood. The blood hosts can vary between, for example, humans, dogs, swine, chickens and rodents. This behavior increases the infection risk for each host as the diseases can be transferred. Feeding on different hosts could be a fitness advantage due to the varying composition of the blood (Harrington, Edman et al. 2001). The genome of *A. aegypti* has approximately 1.35 Giga base pairs (Gbp) and is therefore five times larger than the approximately 278 Mega base pairs (Mbp) *Anopheles gambiae* (Malaria vector Mosquito) genome (Nene, Wortman et al. 2007). These sequenced genomes can be used for further studies of the antennae and the associated genetic background of sensory organs for host-feeding. Knowing, for example, which proteins are involved could help us to determine molecular pathways involved in chemosensation.

### 1.3.2 Transmitted Diseases

#### 1.3.2.1. Yellow-fever

Yellow fever is a viral infection caused by the yellow-fever virus which is an RNA virus found in the family flaviviridae. Flaviviruses are positive-sense, single-stranded RNA viruses that replicate in the cytoplasm of infected cells (Monath 2001). Typical symptoms are for example renal failure, cardiovascular collapse and bleeding. The diagnosis of yellow-fever illness is variable and can range between undiagnosed to liver and kidney failure (Monath 2001). *A. aegypti* is the primary vector of yellow-fever, which is most commonly prevalent in the tropical part of South America and Africa. The mosquito can spread the disease quickly by feeding on several blood hosts. The first evidence for *A. aegypti* being the vector was found in 1900 by James Carroll. Carroll has proven the disease by filtering blood cultures of yellow-fever patients and then injecting the filtrate in three patients with the result that all volunteers died. Therefore, Carroll suspected that yellow-fever was caused by submicroscopic organisms, similar to the previously discovered foot-and-mouth disease (Logue 1995).

Between the years 1990 and 1999 11,297 cases of yellow fever were reported (9358 cases in Africa, 83%) with 2648 deaths. In South America yellow-fever existed in the Amazon region

and the surrounding areas, and between 1990 and 1999 there were 1939 cases and 941 deaths. From 2000 onwards, more yellow fever outbreaks have happened throughout Africa (in e.g. Guinea, Sierra Leone) and the disease is therefore still present worldwide (Monath 2001). In summary, yellow-fever is ‘the recurring plague’ (Tomori 2004). Tomori justified his statement by the fact that annually approximately 200.000 people still suffer and 30.000 die from the disease, despite the availability of an effective vaccine (Tomori 2004). Overall, successful prevention methods are insecticides, repellents or the cost-effective way of preventing standing water (like closed plastic containers for water storage) (Zettel and Kaufman 2012).

#### 1.3.2.2. Dengue Fever

*Aedes aegypti* is also known to carry dengue fever, mainly found in tropical as well as subtropical areas (Zettel and Kaufman 2012). Dengue fever is also caused by a flavivirus. The outbreak is commonly known as the ‘break-bone fever’, arising from the resulting pain (Zettel and Kaufman 2012). New studies show that millions are affected with recent epidemics of Dengue and between 250,000 and 500,000 people worldwide suffer from the dengue consequences, with a fatality rate of 1 to 5% (Harrington, Edman et al. 2001).

#### 1.3.2.3. Zika Virus

The Zika virus disease also belongs to the flavivirus family and is transmitted by *Aedes* mosquitos (mainly *A. aegypti*). The mosquito-borne disease was first identified in 1947 in Uganda in monkeys and moved to Africa, America, Asia and the Pacific Region. Symptoms are mild fever, skin rash, muscle and joint pain that normally last 2 to 7 days (WHO 2016). Another recent concern with the Zika virus is the increased microcephaly in fetuses causing abnormally small brains (Mlakar, Korva et al. 2016). In 2013 and 2015, outbreaks occurred in French Polynesia and Brazil. The prevention of mosquito breeding sites is essential to reduce the Zika disease as no vaccine is so far available (WHO 2016).

## **1.4 Host-seeking in Ectoparasites**

### 1.4.1 Host-seeking of Bed Bugs

Bed bugs need blood to produce eggs (Reinhardt and Siva-Jothy 2007), and need to locate the potential source by sensory cues. The main sensory organ for host detection is the antenna. Host location in blood-sucking insects can be divided into three parts: appetitive searching,

orientation towards the host (by host stimuli) and host contact (Reinhardt and Siva-Jothy 2007). Overall, the present research focused on the sensory cues used in orienting towards the host.

A study from Kemper in 1929 showed that bed bugs can perceive warmth, but it was postulated that it is not necessary for host-seeking (Kemper 1929). Another study of Rivnay revealed a positive thermotactile behavior of bed bugs. Rivnay showed that bed bugs are positively thermotactic by reacting to a human hand at a maximum distance of 4 cm and repelled by temperatures over 40 °C. However, reacting to a 4 cm distance postulates that the bed bugs perceive warm air (convection). Rivnay also stated that odor can sometimes attract and repel (Rivnay 1932), therefore it remains controversial if the hand is only detected by the warmth. Furthermore, Marx also postulated that bed bugs use temperature cues for host-seeking. Therefore, experiments were done with starved bed bugs in an arena containing a beaker with warm water. Setting the water temperature between 35 to 45 °C showed no attraction of the bed bugs. However, with a hotter water temperature between 50 to 60°C the bed bugs were attracted. These findings let us postulate that conduction or convection of heat attracted the bed bugs. A possible explanation for conduction is the fact that the floor temperature was raised by 3°C and therefore attracted the bed bugs. Another possible reason could be that convection accrued at the warm beaker's surface, which attracted bed bugs (Marx 1955). Summarizing Marx's study can be said that bed bugs were attracted by temperature but possibly due to conduction or convection. Another research team (Anderson, Ferrandino et al. 2009) investigated the best artificial versions of host cues as attractants for a bed bug trap. The results showed that a trap with CO<sub>2</sub> (50 to 400 ml/min), heat (37.2 – 42.2°C) and several compounds (33 µg proprionic acid, 0.33 µg butyric acid, 0.33 µg valeric acid, 100 µg octanol and 100 µg L-lactic acid) attracted more bed bugs than each cue alone (Anderson, Ferrandino et al. 2009). Another study was done by Aboul-Nasr, assuming that temperature is important in the bed bug host-seeking. The study showed that bed bugs searching for food exhibit a probing response when they are close to a warm object or warm air currents. Aboul-Nasar suggested that the temperature responses were elicited by the "warmth of the air" - by convection (Aboul-Nasr and Erakey 1967). Overall, combining this research result with those mentioned shows that temperature is an important cue for host finding.

Olson found that the bed bug antennae contain many sensilla as grooved pegs, smooth pegs and immersed cones. Further, Olson found cuticular pores with two pegs with an assuming olfactory function and smooth hairs that look like gustatory sensilla. So far, these findings revealed a small amount of the possible functions of the antennae's sensilla. Furthermore, Olson showed

that bed bugs aggregation behavior after feeding is mediated by several sensory organs located at the antennae. The removal of the distal half of the antennae significantly reduced aggregation behavior of bed bugs (Olson, Moon et al. 2014). Overall, these results show that the bed bug antennae need to be equipped with several sensilla which are, for example, used in off-host aggregation behavior and potentially also sense human cues. However, Wang found a pair of small ‘sensilla coeloconic’ (Fig. 2) in *Anopheles gambiae* mosquitos at the distal tip of the antennae containing neurons that respond to a temperature rise (Wang, Qiu et al. 2009). The morphology of the immersed cones (Fig. 9.1, materials and methods) was similar to the coeloconic sensilla of the mosquitos (Fig. 2), therefore we suspected that these structures might be involved in host-seeking.

#### 1.4.2 Host-seeking of Mosquitos

Similar to bed bugs, mosquitos also utilize antenna to locate hosts in the environment for blood meals. After locating the host, mosquitos navigate towards the host in order to feed blood.

A study from Takken and Knols (1999) states that odor-mediated behavior of *Anopheles gambiae* and *Anopheles funestus* is responsible for many female behaviors. Moreover, olfactory cues are the most important group of external stimuli and the associated receptors are located on the antennae and maxillary palpi (Takken and Knols 1999). The resulting behavior can be influenced by physiological conditions, which is determined by age, size and nutrition status (Klowden, Bock et al. 1996). However, once the threshold value has been reached, a predetermined series of behavioral steps occurs. For example olfactory cues elicit in female mosquito several behaviors like sugar-feeding, host-seeking and oviposition. Examples for important host odors are, for instance, several human skin residues (natural odor), CO<sub>2</sub>, lactic acid and 1-Octen-3-ol which were found to elicit behavioral and electrophysiological responses in *A. aegypti* mosquitos (Takken and Knols 1999). Further it can be suggested that more receptors for other host cues are located at the antennae. Like bed bugs, mosquitos are able to respond to CO<sub>2</sub> at concentrations of 0.04 – 0.6%. Costantini suggested that an odor-baited entry trap efficiency was increased by using CO<sub>2</sub> in combination with human odor (Costantini, Gibson et al. 1996). Other studies also showed that *A. aegypti* is attracted by a range of carboxylic acids (Carlson, Smith et al. 1973), many of which are present in human skin compounds. The production of acids varies between humans, as the production is linked to the metabolic activity of the microorganism. This could explain the different attractiveness of different individuals to mosquitos (*A. gambiae*).

Geier and Boeckh found that skin extracts attracted 80 to 90% of the mosquitos within 30 seconds. L-(+)-lactic acid was identified in the skin extract (Geier and Boeckh 1999). Overall, it was shown that skin residues (odors) and CO<sub>2</sub> are important cues for host-seeking. Furthermore, Peterson and Brown showed that a warm object is more attractive to female mosquitos than the cooler object. The experiment was conducted with a heated-up ball that was either heated up to 100 to 110°F (37 - 43°C) or to 80 to 90°F (26 - 32°C). Petterson and Brown observed that the mosquitos touched the warm ball. This could mean that the mosquitos were attracted by the warm air currents (convection) of the ball (Peterson and Brown 1951). Besides, the role of convection in mosquitos' attraction to human skin was studied by Khan. For the experiment, a human palm was tested at different heights in a tower with sides out of nylon net. The results show that the number of mosquitos navigating to the hand decreased by blocking the convection in the tower. Khan states as well that heat acts through generating convection currents. This statement is not correct as heat can be transferred in three ways: convection, conduction and radiation. Therefore, the experiment showed that mosquitos are able to use convection in order to find a host (Khan, Maibach et al. 1968). Moreover, Wang found the anopheline homolog of the transient receptor potential (TRP) channel (agTRPA1) in distal antennal sensory structures. These sensory structures express agTRPA1 and respond to temperature gradients. The expression studies of agTRPA1 in *Xenopus* oocytes supported the finding that agTRPA1 is involved in the response towards temperature gradients (Wang, Qiu et al. 2009). However, Wang examined a molecular basis for heat sensitivity in *An. gambiae* mosquitos and found a pair of small 'sensilla coeloconic' at the distal tip of the antennae containing neurons that respond to a temperature rise. McIver also described Sensilla coeloconica (pegs in a pit) in *A. aegypti* and *Culex pipiens*. The sensilla coeloconica were previously misidentified as campaniform sensilla. The sensilla coeloconica are located at the distal tip of the *A. aegypti* antennae, on segment 13. The distal end is free of trichoid and basiconic sensilla but each sensillum can be seen as a peg (Fig. 3) located in a pit (Fig. 2, (p)) (McIver 1973). McIver was able to measure the coeloconic sensillum dimension of *A. aegypti*. The peg is approximately 2.5µm long, 1.4µm deep 0.35 µm in diameter (McIver and Hutchinson 1972). The coeloconic sensilla ('peg in a pit' sensory organ) were also observed in other mosquito species. McIver was able to show that both sexes of *Deinocerites cancer* have coeloconic sensilla at the distal dips of the antennae. The study revealed that each peg contains a single pit with similar fine structures to the previously described coeloconic sensilla in *A. aegypti* (McIver and Siemicki 1976). Coeloconic sensilla are not restricted to mosquitos as another study from Steinbrecht showed several sensilla in pits in different insect taxa to be

olfactory sensilla. For example, pits were found in locusts (*Lucusta migratoria*), the greenhouse whitefly (*Trialeurodes vaporariorum*), in the flower chafer *Potosia cuprea*, the Carolina sphinx moth *Manduca sexta* and the fruit fly *Drosophila melanogaster* (Steinbrecht 1997). However, Peterson and Brown's study showed that female mosquitos were attracted by heat transferred by convection (Peterson and Brown 1951) and Khan found that blocking convection decreases the number of mosquitos navigating to a human palm (Khan, Maibach et al. 1968). Combining these studies with the bed bug papers from Aboul-Nasr assuming that convection elicits a host-seeking response in bed bugs (Aboul-Nasr and Erakey 1967), shows the important impact of warmth as a host cue. Additionally, Wang examined a molecular basis with the agTRPA1 channels to respond to temperature gradients. Overall, in all studies it was shown that heat is perceived as convection, however we suggest that the insects use radiation for host-seeking (see results).

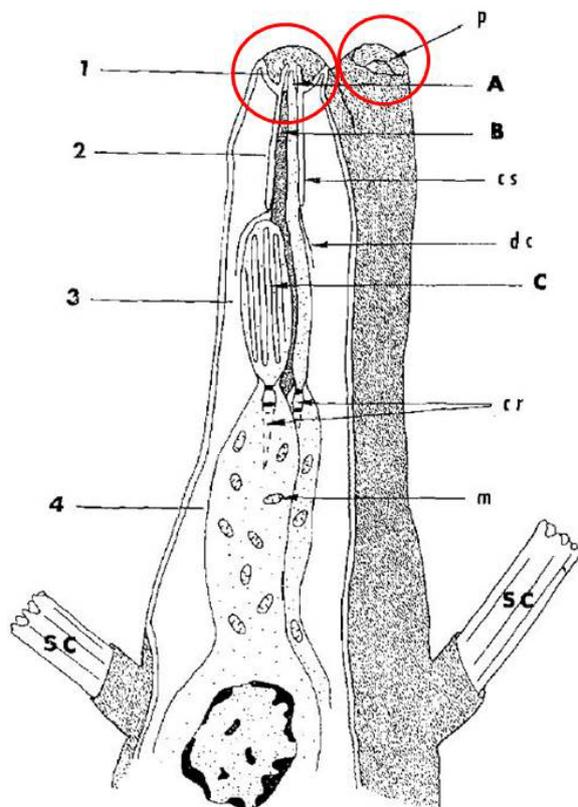
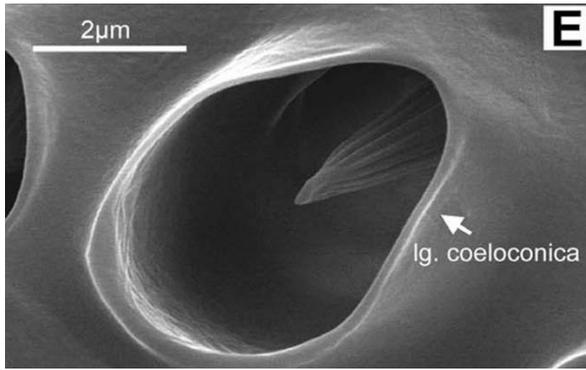


Fig. 2 **Diagram of the tip of the female *Aedes aegypti* antenna**

The diagram shows the tip of the antenna with the associated neurons in a 'cut-away view' of one 'sensillum coeleconicum'. *A. aegypti* has two sensilla coeloconica (red circles) located at the tip of the antennal segment 13. The neuron **A** extends into the peg (**p**), neuron **B** terminates near the base of the peg (**p**) and neuron **C** is located in the outer segment of the dendrite. The sensillum looks like a peg located in a pit (**1**, **2**). The further abbreviations are: cuticular sheath (**cs**), dark inner component (**dc**) of the cs, ciliary region (**cr**), mitochondria (**m**), nucleus (**n**) of the neuron and the sensilla chaetica (**SC**).

Drawing modified from (McIver 1973)



**Fig. 3 Scanning electron microscope (SEM) picture of the coeloconic sensilla of *An. gambiae* and *Anopheles quadriannulatus***

This graph shows the large coeloconic sensillum (the peg in the pit) with the opening (pit) and the peg inside. This SEM was from an *Anopheles* mosquito organ, but the organs of *A. aegypti* look similar (see Fig. 2, red circles)

Picture source: (Pitts and Zwiebel 2006)

## 1.5 Infrared

The previous literature review stated that mosquitos and bed bugs, use temperature as a host cue to locate a feeding source. Until now, literature has assumed that heat is perceived by convection. This statement is correct; however, heat is generally transferred in three ways: convection (warm currents), conduction (heat transfer through contact) and radiation (transfer of energy by electromagnetic waves). This study examines the possibility of mosquitos, as well as bed bugs, using IR-radiation in host-seeking. Infrared is invisible radiant energy that has longer wavelength than visible light (NASA 2010).

### 1.5.1 Electromagnetic waves

Electromagnetic energy arises from the fact that for instance a changing magnetic field will induce a further change in another magnetic field. This change in the magnetic field will form electromagnetic waves. These waves can be classified in different groups (ranges of frequencies). The full spectrum ranges from very long radio waves to short gamma rays, of which the human eye can only detect a small part – the visible light (400 to 700 nanometer). However, IR is also part of the electromagnetic spectrum (details see 1.4.3 IR radiation). Over 200 year ago, in 1800, William Herschel discovered IR while conducting an experiment in which he measured the temperature between the colors of the visible spectrum. The results Herschel observed were the following: First, a temperature increases from the blue to the red spectrum and second, that a warmer temperature outside of the red end of the visible light spectrum exists - the infrared (NASA 2010).

### 1.5.2 IR Radiation

The IR radiation, as mentioned before, is part of the electromagnetic spectrum and consists of longer wavelength than *e.g.* visible light (0.4 $\mu$ m- 0.7 $\mu$ m). The exact spectrum definition varies but it can be said that it ranges from 0.76-1000 $\mu$ m. Furthermore the IR spectrum is divided by wavelength characteristics in three different categories: near-IR (NIR, 0.76-2  $\mu$ m), mid-IR (MIR, 2- 4  $\mu$ m) and far-IR (FIR, 4-1000  $\mu$ m). In more details, IR radiation has a spectral and directional dependence as an electromagnetic wave. Spectral dependence means that the emitter radiates with different wavelength and each emitted wavelength/band depends on several factors like, for example, the temperature of the emitter. When the waves hit an object, directional dependence comes into action but the physics behind that is more complicated. However, the maximum radiation of the wavelength is determined by the temperature of the emitting source. This relationship is described by the basic law of blackbody radiation in *e.g.* the Planck's law (the calculate amount of heat-flux), Wien's displacement law (wavelength

where radiation by blackbody reaches maximum emissive power) and Stefan-Boltzmann's law (total radiated energy from a blackbody at given temperature). Planck's law displays in more details the spectral distribution of the radiation (Fig. 4) from a blackbody source that emits 100% IR radiation at a given temperature. Planck's curve (Fig. 4) shows that the level of emissive power rises with an increase of temperature and that the wavelength of the maximum emissive power shifts to shorter wavelength (x-axis Fig. 4). Therefore, Planck's law can be used to estimate for instance the amount of radiative heat flux when a specific surface temperature of a heating element is known (Pan and Atungulu 2010).

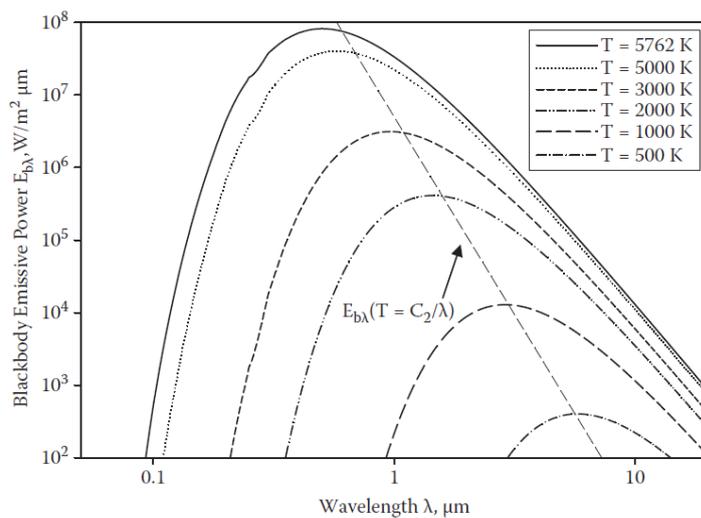


Figure 4 **Planck's Curve with the Blackbody emissive power spectrum**

The graph depicts the blackbody emissive power (y-axis) at different temperatures measured in Kelvin according with the wavelength (x-axis). 0°C is equal to 273.15 K, therefore is e.g. 500 K = 226.85 °C.

Source of graph: (Pan and Atungulu 2010)

### 1.5.3 IR Thermography

It is important to know that IR cannot be detected by human eyes, but that modern technologies made it possible with, for example, IR cameras. The visualizing technology behind Infrared (IR) cameras is called 'Thermography', which allows measuring the IR radiation without getting in contact with the emitting object. The technology behind the used IR camera (FLIR Model E8) is simple as the IR radiation (= electromagnetic waves) is focused over a lens on a detector and converted into a visual image showing the temperature. The created visual image shows false-colored temperature gradients. In details, the IR camera calculates the measured object temperature via the measurements of the IR radiance of the object (the emissivity). The measurement to calculate an object's temperature is based on the emission of the target object, the reflected emission from ambient sources and the emission from the atmosphere. Finally, the internal camera software possesses the input into an image with an output error that deviates less than +/- 1 °C (thermal sensitivity < 0.06 °C) (FLIR Systems).

#### 1.5.4 Heat-seeking in other Animals

As mentioned before, the coeloconic sensilla are not restricted to mosquitos. More animals use heat cues to navigate to a host. The following part describes the possibility of an IR sense in ticks, beetles, vampire bats and snakes.

Ticks are known to sense and orient to a warm source. For instance, hungry ticks hold their forelegs higher than they usually do while moving them back- and forward (Lees 1948). As early as in 1937 Krijgsman (Krijgsman 1937) showed that *Boophilus* larvae (blue cattle tick) oriented to the side of a T-shaped tube that was 34°C warm, therefore respond to conduction. Earlier, in 1933, Totze found out that hungry *Ixodes ricinus* orient to a warm tube when they are close (Totze 1933). This statement might not be valid until today but shows that ticks orient to the warmth. For instance, unfed females oriented to the warm tube within 1 cm and wave, while approaching, with their forelegs more active than usual (comparable to Lee's study (1948)). The results showed that ticks respond to a warm tube of 37°C, therefore we suggested that the ticks sense heat as convection. An overall need to be taken into account is that host-seeking is closely related to the nutritional status. Therefore, freshly molted, unfed ticks are not hungry and avoid the warmth as well as fully engorged ticks (Lees 1948). Heat-sensing was also found in *Triatoma Infestans* ('kissing bugs'). Researchers were able to show that the bed bugs orient towards an IR source at 10 cm distance with the temperature similar to their food source temperature at 32°C. However, the bugs showed a significant tendency while orienting towards the warm source with increased antennal movement and locomotor activity (Lazzari and Núñez 1989). A more recent study from Lazzari described the finding of an unusual cave-like organ in the second segment of the antennae of *T. infestans*. The organ shows the same characteristics as thermoreceptors from other animals. The electrical activity of sense cells was recorded with several stimuli as e.g. warmth, CO<sub>2</sub>, lactic and butyric acid. The results showed that all of the three compounds failed to evoke a response, but that the thermal stimuli modified the electrical activity, which revealed a possible thermoreceptive function (Lazzari and Wicklein 1994). Furthermore, a thermoreceptive function was found in more beetle species. Schmitz described IR sensilla in *Melaophila acuminata* (Coelopter; Buprestidae; Jewil beetle). Both sexes of the beetle approach forest fires as their brood depends on burnt wood as larval food (Schmitz, Sehrbrock et al. 2007). The bugs are able to detect an IR radiation source of fires at wavelength from 3 to 10-25µm (Israelowitz, Rizvi et al. 2007). The associated IR receptors sit in two pit organs that are found on the metathorax and each organ consists of about 70 IR sensilla that are closely packed at the bottom of the pit. From outside, only a single

sensillum can be recognized with a dome built with a thin cuticle. Overall, along the two different morphology results the sensillum can either function in the photomechanical principle (cuticular sphere absorbs mid-IR) or as an air-filled cavity inside the sphere (Schmitz, Sehrbrock et al. 2007). More research on the *Melanophila* beetles IR sense was done by Müller who stated that about 70 spherical IR sensillae are located in the *Melanophila* beetles pit organ. The author was able to establish a functional model of the transduction mechanism. The pressure inside the sensillum will be increased by IR radiation which is measured by a mechanoreceptor. Therefore, the radiation will be transformed into a micromechanical stimulus. Müller found out that the external exocuticle was very stiff (thick cuticle) and can therefore act as a pressure vessel which allows the beetles to convert the fluid expansion into a mechanical sensation by heat (Müller, Olek et al. 2008). Schmitz published a further study about the *Rhodnius prolixus* beetles approaching a thermal source by long-wave IR radiation, whereas starved bugs oriented towards short-wave IR. These results show that *R. prolixus* use IR stimuli to find a host (Schmitz, Trenner et al. 2000). Overall, the IR perception was also found in other animals in various ways, such as prey detection, thermoregulation and protection from heat damage. An example for IR sensing in mammals are the vampire bats (*Desmodus rotundus*) which are known to perceive and utilize the presence and changes in thermal energy in the environment (Ebert 2007). The vampire bat is able to detect temperature differences emitted by a warm signal unit at a distance of 8 to 16 cm. The three suggested pits, the IR receptors, sit at the central nose of the bats. The role of the pit organ is not completely known but might be used to detect IR radiation of the bats' preys (Kürten and Schmidt 1982). IR perception in reptiles is as well known in two snake families, the Boidae (e.g. boas) and the subfamily of Crotalinae (e.g. Copperheads). Snakes are able to integrate the visual information that allows them to see different regions of the electromagnetic spectrum. The reason behind that is the biological function to detect homeothermic prey. A brief summary of the snake pit morphology shows that in pit vipers, the IR receptor ('pit') is covered with a membrane and located between the nostril and the eye. The boid snakes have simpler pits but show a wide variety in shape, size and positioning of the pits. The function of the IR sense in pit-bearing boids is similar to a pinhole camera (Ebert 2007) and therefore close to the coeloconic sensilla which we have assumed to perceive warmth in bed bugs and yellow-fever mosquitos. A closer look at the IR detection ranges in snakes gave further insights into possible functions of the pit organs. Therefore, IR detection depends on the threshold (the minimum IR irradiance of an object against the background) at a certain distance. The calculation of the detection ranges in

pit vipers (crotalus; rattlesnakes) spans from approx. 66cm for a mouse (10°C above ambient temperature) to less than 5 cm for a mouse-like stimulus at 37°C (Ebert 2007).

## **1.6 Repellents**

Mosquitos and bed bugs use temperature as a heat cue to locate a host ((Aboul-Nasr and Erakey 1967), (Reinhardt and Siva-Jothy 2007)) and can be repelled by using different repellents ((Thomas, Kihiczak et al. 2004); (KIM, KANG et al. 2005)). Repellents arose due to their characteristics of being low toxic towards humans and animals and having high repellency effects against insects (KIM, KANG et al. 2005). Furthermore, three repellent compounds (of DEET, Cinnamaldehyde, Citronellal) were tested in order to investigate if they affect the insects' heat-seeking behavior.

### **1.6.1 DEET**

DEET, N,N-Diethyl-m-toluamide (IUPAC), is a known insect repellent and was developed and patented by the US Army in 1946 for the protection of military personnel (Jackson 2008). DEET is very effective against several pests, as for instance mosquitos, biting flies, chiggers, fleas, ticks, stable flies and leeches (EPA 1980).

The mode of action of DEET is still unknown but a widely accepted hypothesis has spread that DEET interferes with the detection of lactic acid (Syed and Leal 2008). DEET was reported to inhibit two classes of receptors of the mosquitos antennae, one sensitive to lactic acid and the other inhibited by lactic acid (Dogan, Ayres et al. 1999). Another hypothesis is that DEET masks the olfactory system by decreasing the electrophysiological responses to 1-octen-3-ol (Syed and Leal 2008). However, Syed and Leal (Syed and Leal 2008) suggested that the interaction with lactic acid may not be essential for DEET repellency. Another research group (Lee, Kim et al. 2010) showed that DEET suppresses feeding behavior in *Drosophila* via a mechanism using gustatory receptor neurons. However, DEET is also detected through olfactory receptor neurons (ORNs) via the mechanism of the olfactory receptors (*e.g.* OR83b). It is still unknown if ORNs respond directly to DEET or if DEET blocks the response to attractive odors. Lee's paper suggests overall that avoidance of DEET is based on gustatory and olfactory receptor neurons (Lee, Kim et al. 2010). Other researchers published a study about "comparative efficacy of insect repellents against mosquito bites" (Fradin, 2002) stating that DEET provides long-lasting protection. The repellency effect of DEET is also positively correlated with the used DEET concentration. For instance, 4.75% DEET provided 88.4 minutes of protection. Another example of 23.8% DEET repels mosquitos for over 300 minutes (Fradin and Day 2002).

### 1.6.2 Cinnamaldehyde

Cinnamaldehyde is the main compound in cinnamon leaf oil extracted from the *Cinnamomum cassia* tree. The compound is worldwide used as a food additive and flavoring agent with a pleasant smell and recently known to be a mosquito repellent. A study of Chang suggested that the cinnamon oil might also work as a repellent on adult mosquitos as it is lethal to larva (Yeh, Lin et al. 2014).

Cinnamaldehyde is known to activate TRPA1 (transient receptor potential) channels (Bandell, Story et al. 2004). Another study by Wang identified a homolog of the TRP channel in *Anopheles gambiae* mosquitos that plays a role in heat-sensing of convection (Wang, Qiu et al. 2009). Other studies from Cheng (Cheng, 2003), for instance, show larvicidal activity of cinnamaldehyde on *A. aegypti* larvae. In addition, cinnamaldehyde is the most specific TRPA1 activator that excites a subset of sensory neurons that are highly enriched in cold-sensitive neurons (Cheng, Chang et al. 2003). Moreover, Brandell's study was able to show that the TRPA1 activation elicits a painful sensation (Bandell, Story et al. 2004). Some members of the Ion channels from the TRP family are known to act as temperature sensors (for instance in *D. melanogaster* and *C. elegans*), but it is not known if they can be directly activated by temperature. A further study from Viswanath showed that the *Drosophila* ortholog 'ANKTM1' (cold-activated ion channel) in mammals responds rather to warmth than to a cold stimulus. Another interesting detail the activation threshold of 'dANKTM1' in *Drosophila*, which ranges between 24-29°C, which could have a potential role in heat sensing (Viswanath, Story et al. 2003). However, all these studies suggested a possible effect of cinnamaldehyde on temperature-sensing. We suggested that the coeloconic sensilla might have heat-sensitive channels and therefore the repellent can affect the mosquitos' heat-seeking behavior.

### 1.6.3 Citronellal

The plant oil is found in the citronella genus (Poaceae) and consists of, for example, geraniol, citronellol, limonene and citronellal. The four mentioned components of citronella oil demonstrated inhibition in feeding (limonene, geraniol and citronellol) (Zamora, Klotz et al. 2015), whereas the natural aroma compound citronellal has repellent efficacies against mosquitos (KIM, KANG et al. 2005). In the 20<sup>th</sup> century, the insect repellent was used by the Indian Army and later, in 1948, commercially used in the USA (Zamora, Klotz et al. 2015).

Only a few studies were done on the research of the citronellal repellency effect but it is well-known that Citronella-based repellents protect from mosquitos for approximately two hours. A possible reason for the limited safety is the evaporation that leaves the user unprotected (Maia and Moore 2011). Kwon was able to show that *Drosophila* uses two pathways for avoidance of citronellal. The olfactory coreceptor (OR83b) results in citronellal repulsion and mutation in the TRPA1  $CA^{2+}$ -permeable cation channels results in a defect in avoiding citronellal vapor. However, Kwon was able to show that citronellal directly activates TRPA1 channels in *A. gambiae* (Kwon, Kim et al. 2010). Therefore, we suggested that citronellal also affects the *A. aegypti* heat-sensitive channels with the effect of an inhibited host-seeking.

## **1.7 Question**

Mosquitos and bed bugs use several sensory host cues to locate a blood source. This study focused on heat as a host cue. The reason for the focus on heat is that a co-worker found that ticks can perceive warmth. Therefore, we postulated that the insects can not only use convection to find a host but also radiation. The ticks' morphology of the sensory organs proposed a possible structure with a peg in a pit (coeloconic sensilla) being involved in host-seeking. Therefore, we suspect that the window in the sensillum (pit) can recognize the direction of the heat, i.e. the coeloconic sensilla can be a directional sensor as the window restricts the field of view. This hypothesis suggests that ticks can locate the host by using IR radiation to seek the warm host. This hypothesis provided evidence that the same host-seeking behavior could be used by mosquitos and bed bugs. Furthermore, the action of three repellents (DEET, Cinnamaldehyde and Citronellal) was also tested to find out if the compounds affect the host-seeking behavior of mosquitos. Therefore, experiments were done using a warm, IR-emitting source to test the host-seeking behavior of bed bugs (*Cimex lectularius*) and mosquitos (*Aedes aegypti*). The warm source emitted IR radiation, which we presented the insects' as the host cue. In order to emit IR, two thermoelectric cooler elements (TECs) were used to provide for the two ectoparasites a warm (40°C) and cold (22.8°C, room temperature) source.

## 2. Materials and Methods

### 2.1 Bed Bugs

Bed bugs (*Cimex lectularius*) were obtained from the In-house Insectary (BASF, RTP, NC, USA). The strain was established several years ago and is maintained at 25 °C (at ~50% humidity) with a 14:10 h Light:Dark (L:D) cycle. After the bed bugs were transferred in the lab, the light:dark cycle was reversed to a 14:10 h L:D cycle, in which day- and nighttime were interchanged in order to test the nocturnal behavior of bed bugs during the day. The bed bugs had a minimum of 96h (4 days) to adapt to the reversed cycle. The cycle consists of a 'lighted' day cycle with a full spectrum lamp (NFK Lite Mfg. Co. Ltd, Model No.: 4026, Rating: 120V, 60Hz, 0.41A; lightbulb full spectrum: FML 27W 6500K, 1400 Lumen) and a dark, non-lighted night cycle. All bed bugs were starved before arrival and finally tested 7 – 18 days after being put on the reversed L:D cycle. The insects were held in a plastic container with paper as harborage and a screen for air exchange. The smaller jar was covered by bigger one to prevent escape.

#### 2.1.1 Assay Protocol

An arena for the bed bugs was built with 19 mm (3/4 inch) thick plywood with maximum interior dimensions of 10 X 10 X 100 cm (Fig. 5). The inside was painted with a low reflective paint (acrylic latex paint with matte finish). This paint reduced reflection to a minimum, which was verified with a thermal infrared camera (FLIR Systems, Model FLIR E8). Thermoelectric coolers (TECs) (Vuemetrix, Vue-TEC Developer's Kit with Windows Application (WinVue), Fig. 7 II and 8) were used to create 10cm x 10cm temperature-controlled surfaces at the ends of the arena, which could be moved closer together to create a shorter arena. The TEC's surface is a blue anodized aluminum plate that efficiently emits infrared radiation. The temperature on each TEC was checked with a thermocouple thermometer (Physitemp Instruments Inc., Model BAT-12, New Jersey, USA, see (III) in Fig. 7) inserted into the aluminum plate. The thermometer temperature measurement was 0.1 degree Celsius accurate. In one out of three experiments, aluminum foil was adhered with a permanent glue stick to the TEC's surface with the shiny side out in order to provide a warm surface with very low emissivity but high reflectivity. The surface temperature, as verified with a thermocouple thermometer, was 37 °C, but it appeared to the IR sensor of the camera to be at the ambient temperature because it reflected the IR emitted by the surroundings (Fig m6).

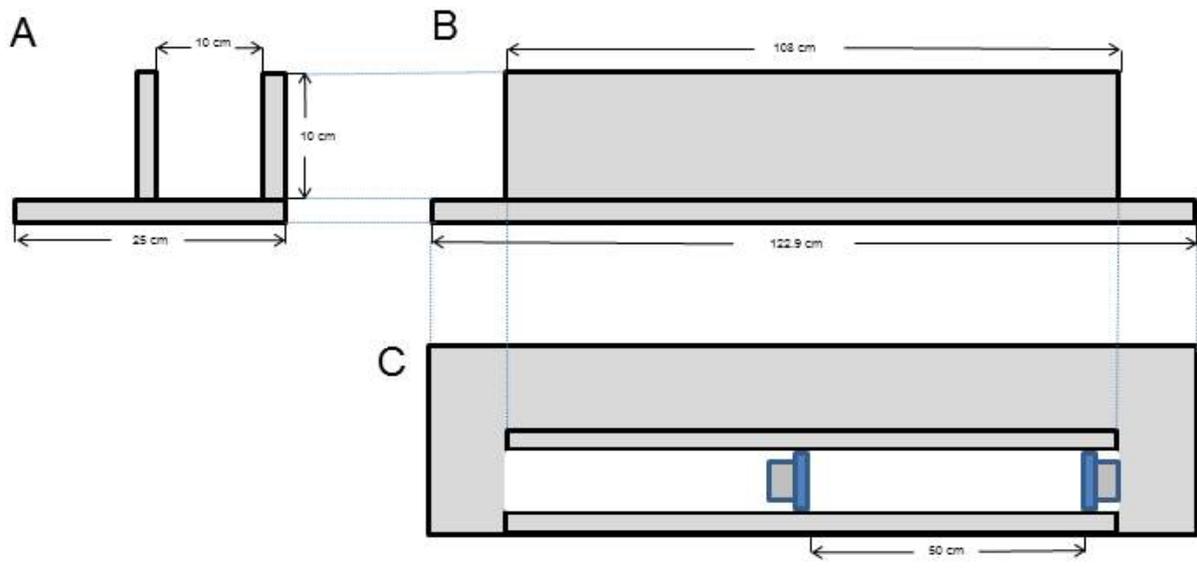


Figure 5 Overview of the bed bug arena

The graph shows the overview of the construction and measurements of the bed bug arena. **A** displays the side view, **B** the view from the front and **C** the top view.

The dual choice experiments (e.g. 22.8 °C (room temperature) vs. 37 °C) were performed in a symmetrical elongated arena (Fig. 5c and Fig. 6). The 50 cm symmetrical arena was divided in the three parts (Fig. 6): the cold side (choice: bed bugs did not orient to the warm side, definition: 0-20cm away from cold TEC), the center (choice: no response, definition: within 20-30 cm away from cold TEC) and the warm side (choice: bed bugs found warm side, 30-50 cm away from cold TEC). The bed bugs were tracked over time and the data evaluated which choice they made. The arena was covered throughout the behavioral assays with a ¼" top (Plexiglas) to allow filming but avoid interference by Infrared emitted by the observer.

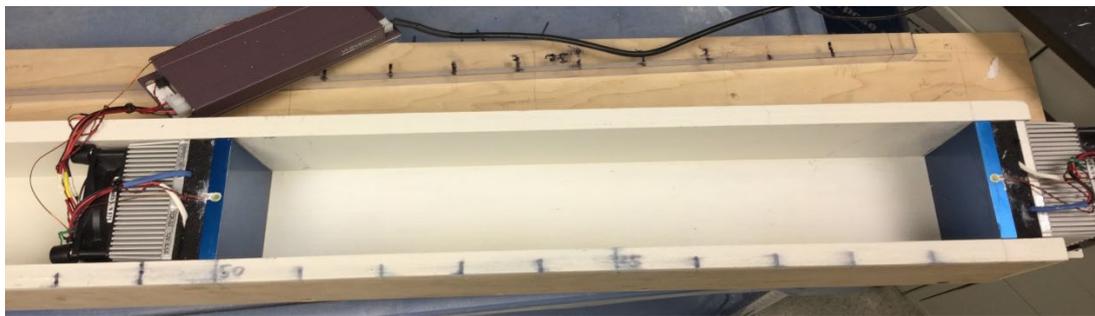
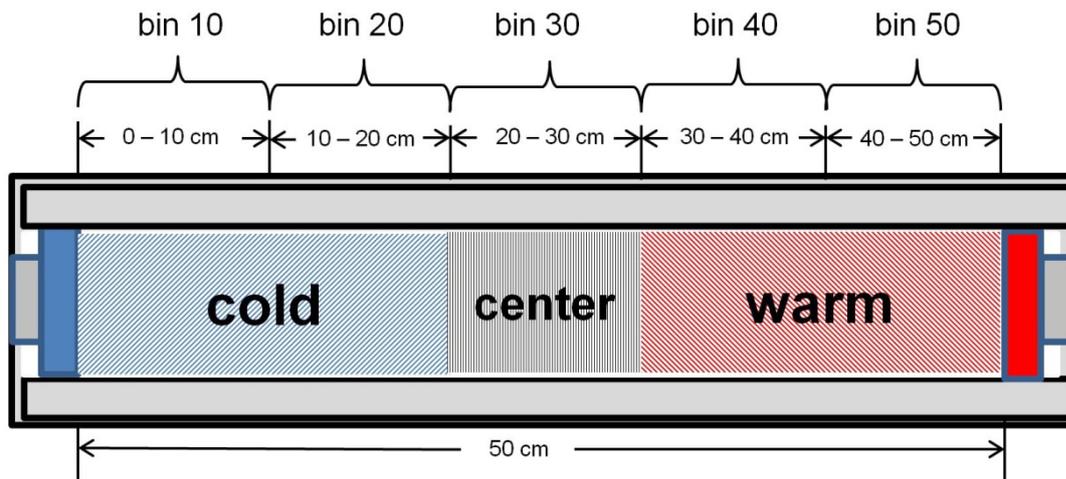
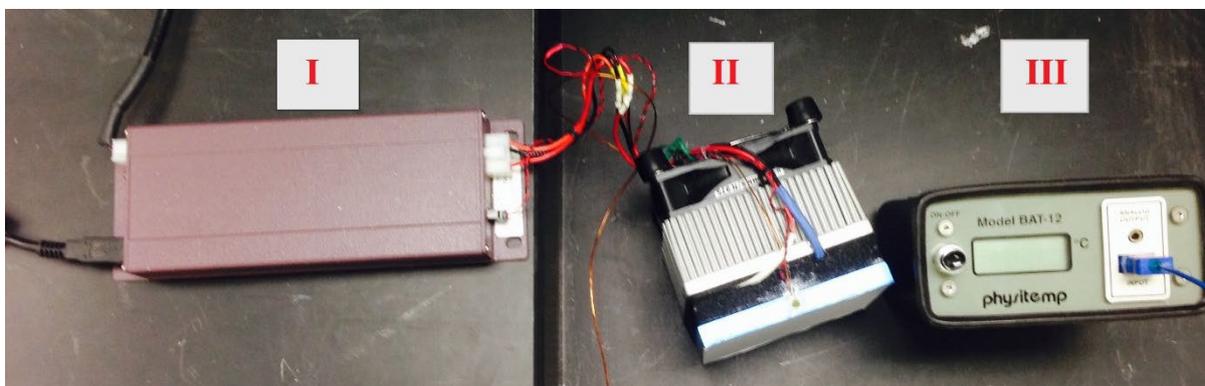


Figure 6 Detailed sketch and original setup of bed bug running chamber

This graph depicts the sketch (above) and the original setup (below) of the bed bug running chamber details (top view Fig. 5 C). The chamber was divided in three parts: the cold (blue), center (black) and warm part (red). The blue module at the far left side in the chamber is the cold TEC, whereas the opposite TEC is the warm side. The first step for the data collection was that all data was arranged in a frequency table (Fig. 12). Here, the raw data was clustered in bins, in which *e.g.* bin 10 depicts the distance from 0-10cm away from the cold side. Second, bin 10 and bin 20 were summarized into the cold category, bin 30 into the center and bin 40 and bin 50 to the warm side (30-50cm away from the cold side). However, the three categories (warm, center, cold) are highlighted in the sketch with the associated colors (red, black, blue). The three colors were used for all further graphs.

A



B

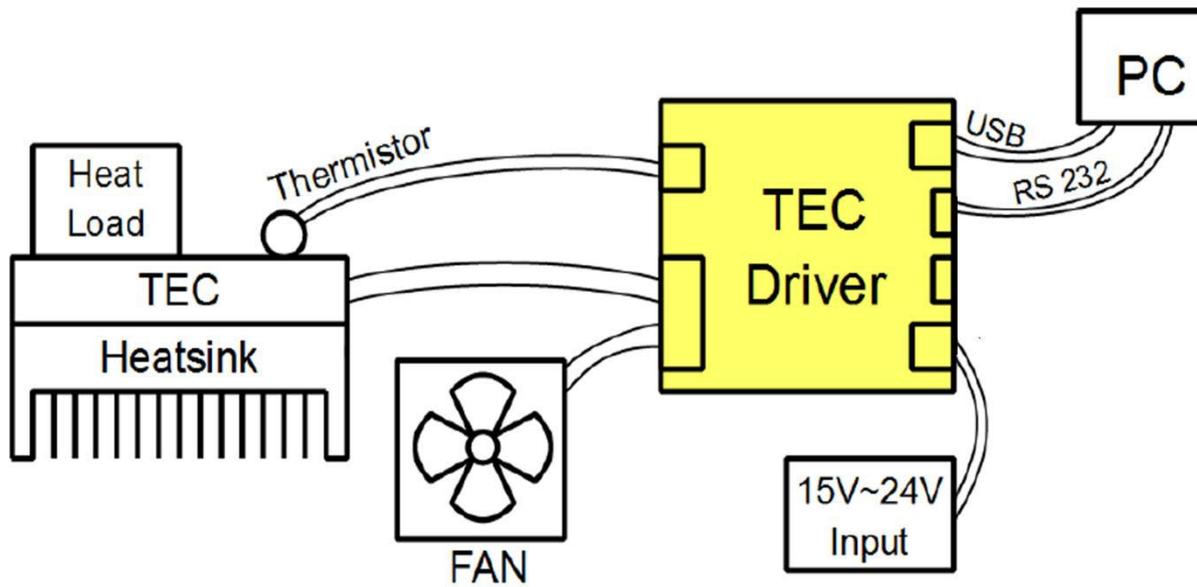
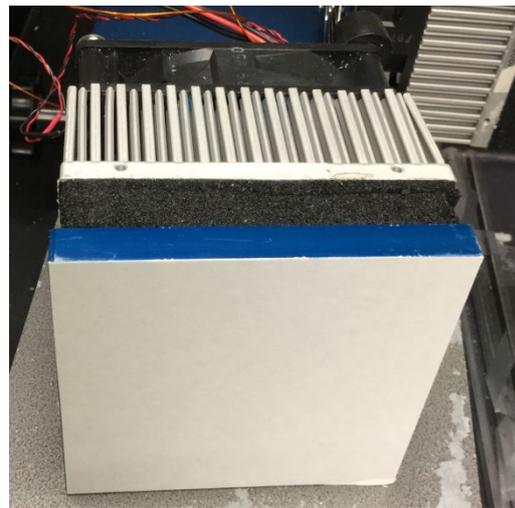


Figure 7 Overview of the Thermoelectric cooler (TEC).

**A** depicts the current setup with the (I) driver (S/N 975), the (II, detail Fig. 8) Heatsink/cold plate (SN: TCP08153) and a (III) thermocouple thermometer (Physitemp Instruments Inc., Model BAT-12, New Jersey, USA). **B** illustrates the circuit diagram of the TEC setup with the computer connection (USB) of the TEC Driver, the cooling fan and the cold plate (here referred as TEC Heatsink). The double lines symbolize the individual connections between the parts and the driver.



A



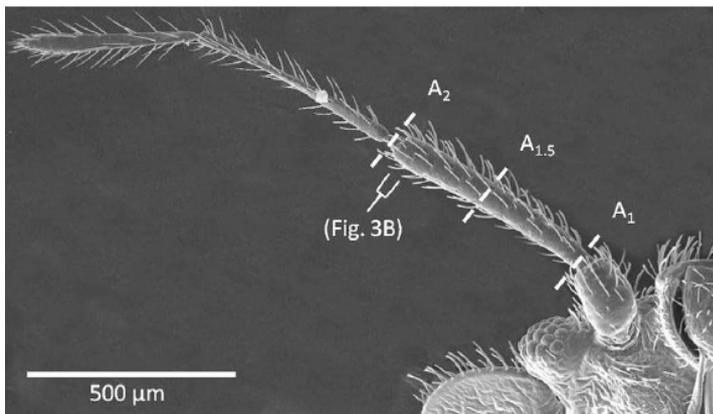
B

Figure 8 Thermoelectric cooler (TEC)

The TEC has a blue anodized coating (A) that efficiently emits IR (emissivity near 1). For the experiments, a white printer paper (B) was glued over the blue anodized coating to enhance the contrast for filming, without decreasing emissivity (checked with IR camera).

### 2.1.2 Behavioral trials

One TEC was set at 37 °C (warm) and the other at 22.8 °C (cold, room temperature). The air temperature did not vary above ambient more than a few mm from the warm plate. In order to control for possible side preference, the location of the warm TEC was reversed every 3 trials. For each trial 2 bed bugs were simultaneously released (at the 25 cm mark – the arena’s center) and tested for 10 minutes without any breaks. The experiments were recorded with a camcorder (Bell and Howell DNV16HDZ, Full 1080p HD Infrared Night Vision Camcorder). The chamber was cleaned after every trial with 70% Ethanol to avoid traces as e.g. pheromone traces from the previous insects. In some trials, both antennae were amputated distal to the 1<sup>st</sup> segment (A1, Fig. 9). Fig. 9.1 shows the immersed cones (in another paper referred as: coeloconic sensillae) (F, Fig. 9.1) that along the current hypothesis (this thesis) are responsible for infrared sensing. These organs are located on the antennae just proxima to the A2 mark (Figure 9, A2), more specifically at the mark ‘Fig. 3B’ in figure 9.

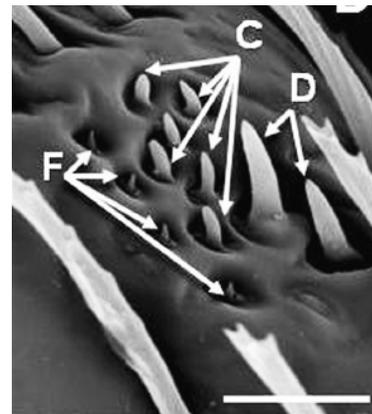


**A**

**Figure 9 Ventral SEM image of Antennal Morphology of *Cimex lectularius***

A ventral view of a bed bug’s head (lower right corner) and one antennae. A1 shows the point where the antennae were cut. The sensory patch that might be responsible for the IR-sense is located at the ‘(Fig. 3B)’ mark. A detailed magnification of the sensory patch is displayed in Figure 9.1 (right side).

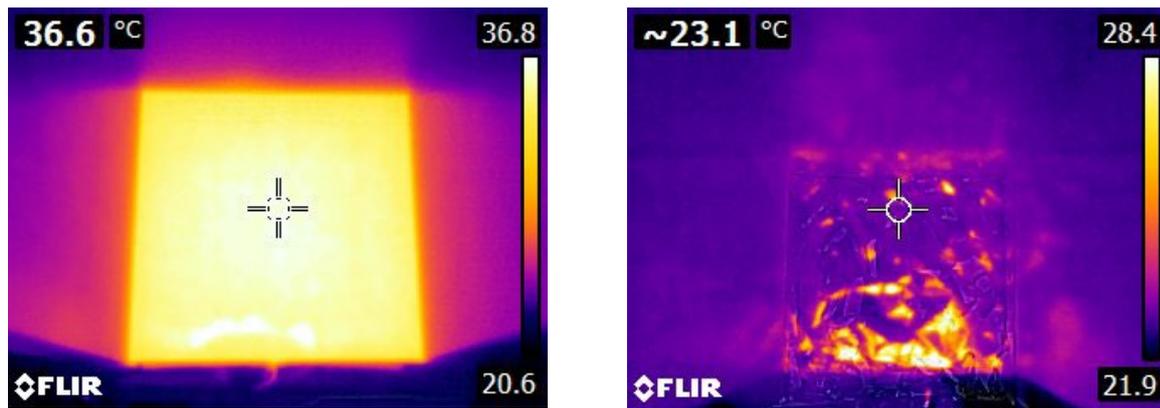
Image source: (Olson, Moon et al. 2014)



**B**

**Figure 9.1 Close-up SEM image of *C. lectularius* sensory patch [Fig. 9 (Fig. 3B)].**

Several hairs can be found at the bed bug sensory patch. However, for this study the cones marked with an ‘F’ are important. F shows the ‘immersed cones’ that along the current hypothesis (this thesis) are responsible for IR sensing (scale bar = 10 μm)



A

B

Figure 10 FLIR Infrared picture of the TECs in the Bed bug arena

Autoscaled false-color thermal infrared images of the TEC surface installed in the chamber. Color scale is shown on the right of each image and the spot temperature in the crosshairs is shown in the upper left. **A** shows the warm uncovered TEC at 37 °C, with the camera recording 36.8 °C. **B** shows the warm TEC element at 37 °C covered with aluminum foil shows a temperature of 23.1 °C.

### 2.1.3 Video and Data Analysis

The HD videos were converted to JPGs (file format: Joint Photographic Experts Group) with ‘The free Video to JPG convertor’ program (Free Video to JPEG Converter, DVD VideoSoft, Digital Wave Ltd.). The conversion into jpeg stacks was done to reduce the size of the HD videos to 1 picture per frame (equal to: 1 picture/second) and to process it further with ImageJ (U. S. National Institutes of Health, Bethesda, Maryland, USA). The JPGs were loaded into ImageJ and directly cropped to show only the arena to reduce processing time. In the next step, all images were converted into a hue, saturation and brightness stack (HSB stack). Out of the three stacks, the brightness stack was duplicated and further used. Finally, the images were transformed to an 8-bit type and processed via the filter option ‘Unsharp Mask’ (Radius=3, Mask=.7, preview checked). After adjusting the contrast, the plugin MTrackJ (ImageJ plugin) was used to analyze the bed bug tracks. The plugin offered the option ‘add’ to create a path throughout the stack. The moving bed bugs were marked at every 30 frames (= 30 seconds) and a path was created (Fig. 11). The distance from the warm TEC in cm was measured for every bed bug over time, to evaluate the navigation behavior.

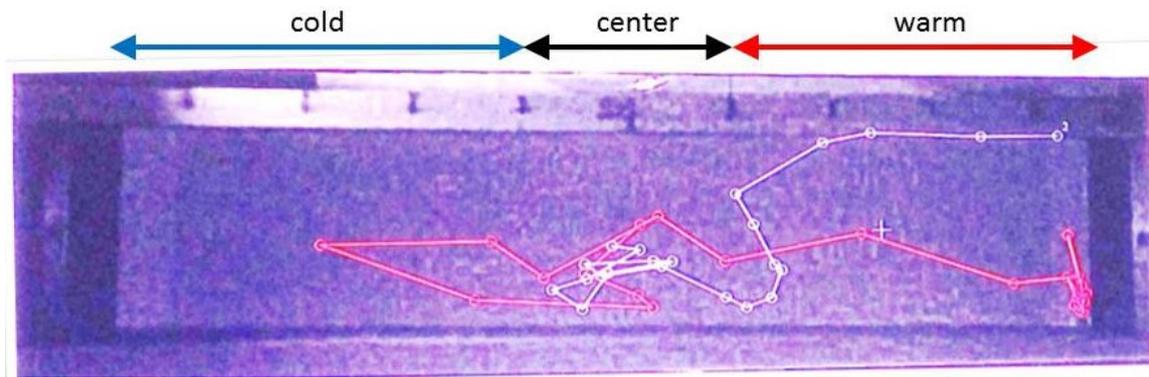


Figure 11 **Bed bugs walking analysis**

This graph shows the walking arena (Fig. 5 C) and the division into three regions (cold, center=start, warm; Fig. 6) during the experiment. Tracks of two bed bugs (red and white) orienting towards and navigating to the warm TEC over 5 minutes are shown. Both bed bugs ended up at the warm TEC. The track analysis was done with the ImageJ plugin 'MtrackJ'.

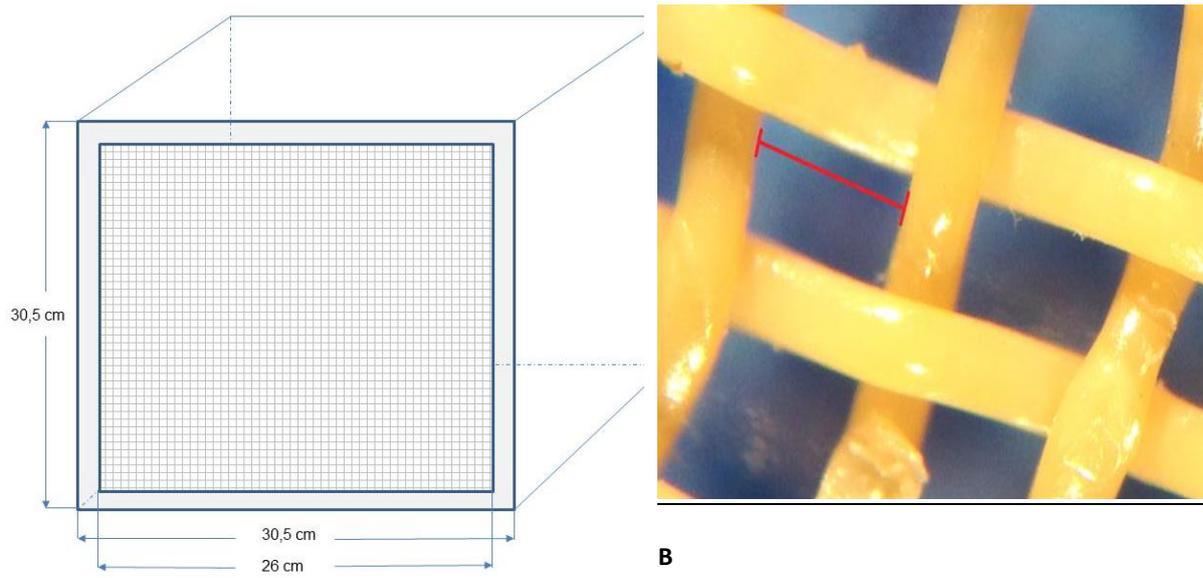
While all trials were recorded for 10 minutes, analysis of only the first five minutes was sufficient. The response to the warm and cold side (warm left vs. warm right, cold left vs. cold right) was transferred into Excel. The data for each side was put in a frequency table and divided into 5 bins. For instance, bin 10 represents the number of bed bugs between 0 - 10cm away from the cold TEC over time. The remaining four categories are: bin 20 (10-20 cm away from the cold TEC), bin 30 = center (20-30cm), bin 40 (30-40cm) and bin 50 (40-50cm). After the bin arrangements, all data of *e.g.* 'warm left' was put in three categories: cold, center and warm. The same was done for the 'warm right' experiment. Finally, the responses to the warm and cold side were averaged. For the scale was chosen that the left side was always the cold TEC and therefore determined as the starting point for the individual track measurements. The averaged responses for the three categories were plotted with Prism 6 (Graph Pad Software, USA).

## 2.2 Mosquitos

Yellow-fever mosquitos *Aedes aegypti* were obtained from the BASF N-CAT (Non-Crop Advance Testing) group based in Research Triangle Park (RTP), NC USA. Eggs were acquired from Louisiana Biologicals (order of 2 mL eggs/month) and were raised in the BASF N-CAT lab at 26 °C with 60 % relative humidity in a light controlled chamber with a 14:10 h L:D cycle.

### 2.2.1 Assay Protocol

The experiments were carried out in screened, aluminum-framed mosquito cages (Fig. 13 A) with a warm (40°C) and cold (22.4 °C) TEC (**TEC: m4**, setup: **m10 A**). The square cages, made by BioQuip Products USA, had external side dimensions of 30.5 X 30.5 X 30.5 cm (length X width X height) and a square inset with amber Lumite™ screens of 26 X 26 cm. The screen mesh size (Fig. 13 B) was approx. 530 µm.



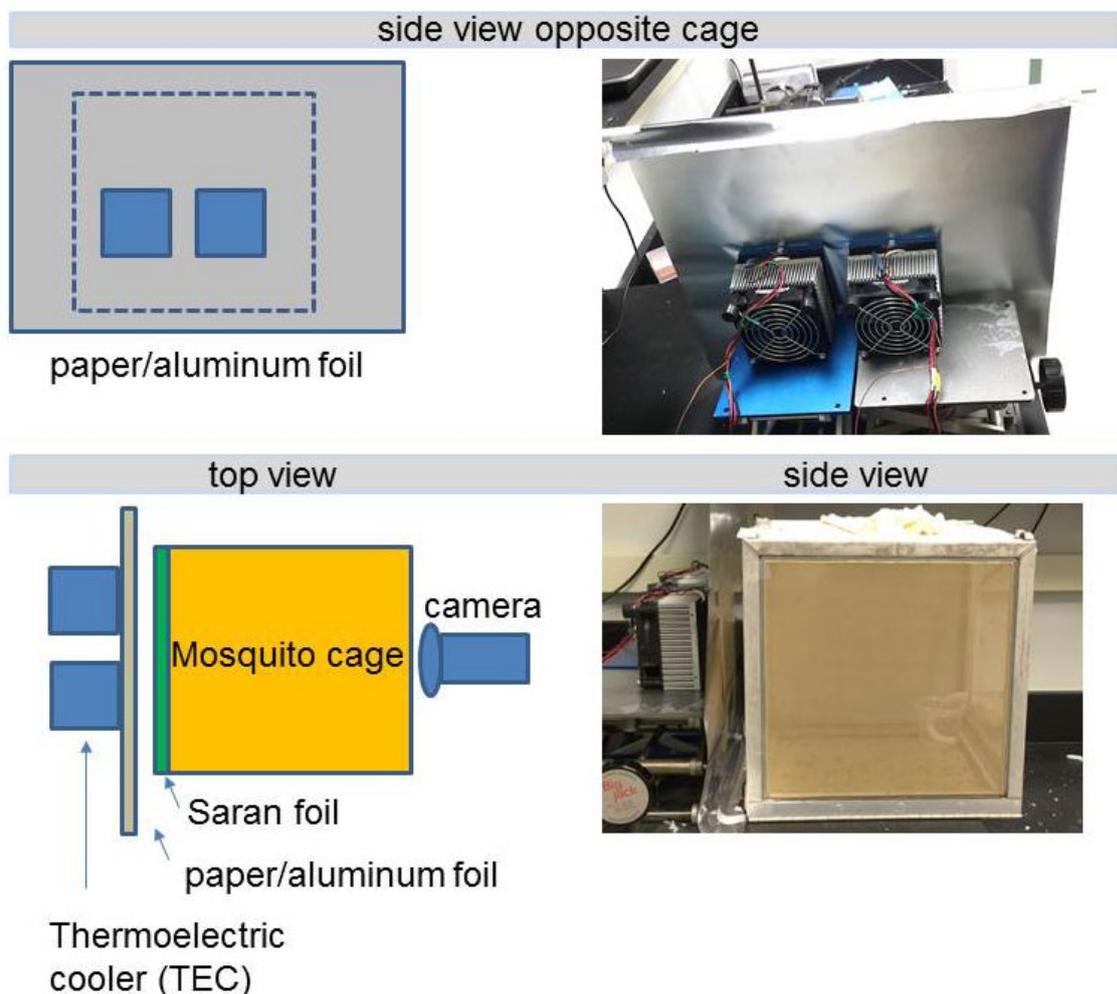
**A**

Figure 13 Overview of the Mosquito rearing and testing cage

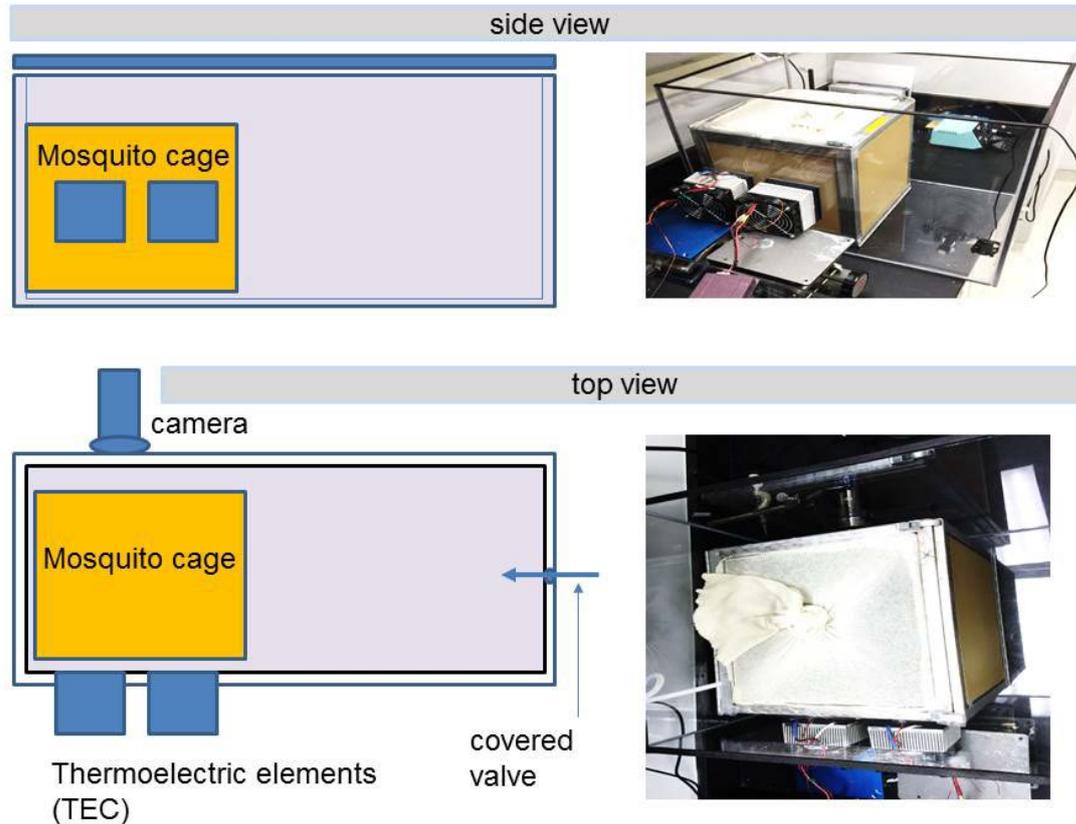
The square cage (A) (BioQuip Products USA) external dimension is: 30.5 X 30.5 X 30.5 cm. The Lumite™ screen (gray shaded squares, Fig. 9A) insets dimension is: 26 X 26 cm. Overall, three sides are screen, one side of cotton mesh sleeve (openable access) and a bottom of solid stainless steel. The mesh width of the screen (B) is approximately 530 µm (B, red bar).

Before the experiments, all cages were stored for approximately 7 days on the laboratory bench. Earlier experiments (data not shown) indicated no thermotactic response under 7 days starvation

(except regular sugar water feeder). All mosquitos were fed throughout the entire time (including experiments) with 10 % pure cane sugar water (Great Value™, Walmart). The light (standard laboratory light conditions) was on from 8:30am to 6pm. For the experiments, two TECs (VUEmetrix Vue-TEC Temperature control system, Developer's kit with WinVue Windows interface, Fig. 8) were used to which either paper (enhances contrast of objects) or aluminum foil was glued (permanent glue stick) on (Fig. 14 A, side view). For other experiments, a closed chamber was used to vaporize compounds (Fig. 14 B).



A



## B

Figure 14 Experimental setups of mosquito behavioral assays

Behavioral assay setup for mosquitos (A) with either aluminum-foil or paper covered TECs. The dashed lines show the test cage borders at the other side. The top view shows the setup overview with the TECs (blue), the paper sheet or aluminum foil (grey), the Saran wrap (green), the mosquito cage (orange) and the camera (blue). The closed arena (B) was used to test the mosquitos' thermotaxis during vapor exposure. The side view graphic shows the installation spot for the two TECs (top view, B). The top view shows the overview with the camera setup, the mosquito cage (orange; details in Fig. 13) and the wax-covered valve (arrow). The wax-covered value is used to introduce the compounds on the soldering iron (vaporizer). The arena's measurements are: 37.5 X 57.2 X 42 cm (length x wide x height) with 90.09 liter volume.

For the mosquitos' thermotaxis experiment two TECs (Fig. 8) were set to 40°C (warm) and 22.8°C (cold) (Fig. 14 A). The temperature was set to 40 °C instead of 37 °C (as in bed bugs) because the Lumite screen partially blocked the IR, reducing the apparent temperature. In order to evaluate the decrease of IR radiation by the screen, the 40°C warm TEC was measured in and outside of the cage with the IR camera (FLIR E8; Fig. 15). The observed TEC temperature viewed from outside the cage was 39.6°C (Figure 15 D) and viewed from inside the cage (with the amber Lumite™ screen) was 35.23°C (average value of three replicates; Fig. 15 A-C). Therefore, the screen decreased the apparent temperature by ~ 4.36 °C to 35.23°C. IR heating

of the screen by the TEC plate can be ruled out as the image with the thermal camera revealed that the source appears as a square with clear edges. For instance, comparing Fig. 15 D (TEC without screen) with Fig. 15A (TEC with screen) shows a similar shape of the square source with sharp edges. If radiative heating would have elevated the screen temperature, then the IR camera would have detected instead of a square object a rather round shaped source. Other experiments replacing the screen with plastic foil (data not shown), with the advantage of high emissivity from 0.95 to 1, were not successful as the mosquitos were not able to land at the plastic foil.

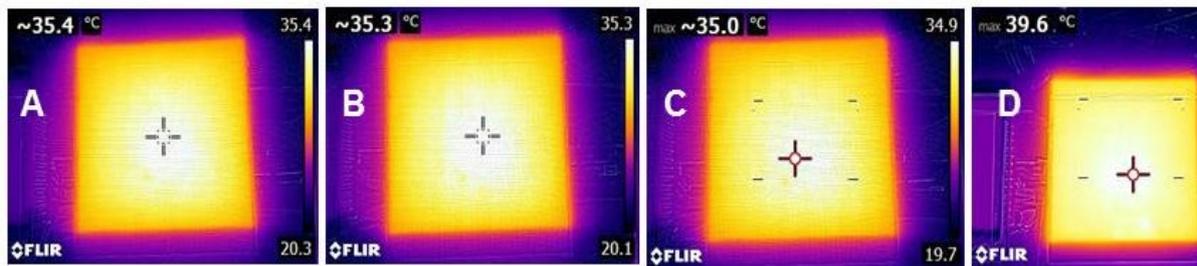


Figure 15 Overview of IR emission of TECs inside and outside of the Mosquito cage

Picture A-C shows three repetitions of IR emission measurement (FLIR camera) from a set 40°C heat source in front of the screen (inside the mosquito cage). The distance of the TEC to the cage screen was 2 cm. The average of 35,23°C from the three temperature measurements (35,4°C (A), 35,3°C (B) and 35,0°C (C)) gives the ‘actual’ temperature (IR radiation) seen by the mosquitos through the screen. Picture D shows the warm TEC measurement of 39.3°C outside of the cage (without screen).

The TECs surface (Fig. 8) is a blue anodized aluminum plate that efficiently emits infrared radiation (emissivity near 1.0) as used in the bed bug experiments. Standard printer paper (Store brand: Staples, Size: 8.5 x 11 inches, weight/sheet: 4.6 g (average of 3 sheets), color: white) was glued (permanent glue stick) over the TECs to enhance visual contrast without losing emission (emissivity around 1; verified with IR camera, data not shown). The TECs temperature was again checked with the thermocouple thermometer (Model BAT-12, Physitemp Instruments Inc. USA, Fig. 7 (III) in Material and Methods - Bed Bugs). The temperature measurement was accurate to within 0.1 degree Celsius with the thermometer embedded in the TEC aluminum plate. Heat sink compound covered the thermometer and provides the temperature transfer between the TEC and the thermocouple. All IR emissions were measured with the FLIR infrared camera (FLIR E8). The IR camera creates a false-color picture to distinguish the warm from the cold objects (Fig. 16 A). Figure 16 A shows the difference between a warm (left square) and a cold (right square) source. However, all object emit IR (see introduction), which depends on temperature. The higher the temperature the more IR radiation

will be emitted. The behavioral experiments were done to test if the yellow-fever mosquitos are attracted to objects at 40°C by detecting it through IR-sensing. After a positive IR-seeking behavior, different distance experiments between the TEC and the cage were tested to determine the detecting range to a warm, IR-emitting source. The four distances between the cage and the TECs were: 1 cm, 2 cm, 5 cm and 10 cm. The 1 cm distance was ruled out after the first experiments as the mosquitos also navigated to the cold sources (due to heat convection). Further experiments were done with blocked IR emissions using aluminum foil at 2 cm, 5 cm and 10cm distance (see Fig. 14 A side & top view). For instance, Aluminum foil (VWR, Ultra-Clean Supremium Aluminum Foil, 18'' x 50', 22.86  $\mu\text{m}$  thickness, Catalog No.:89107-732) has a low emissivity of 0.04. The shiny side of the aluminum foil was facing out to provide a warm surface with low emissivity but high reflectivity. The IR camera did not detect the aluminum-foil covered TEC as warm (yellow/white color) (Fig. 16 B, left square). As seen in figure 16 B, there is no difference in IR emission between the warm (left square, bright yellow) and the cold (right square, blue) source. The TECs surface temperature, as verified with the thermocouple thermometer, was 40°C, but appeared to the IR sensor of the camera to be at ambient temperature because it reflected the IR emitted by the surroundings (*e.g.* experimenter).

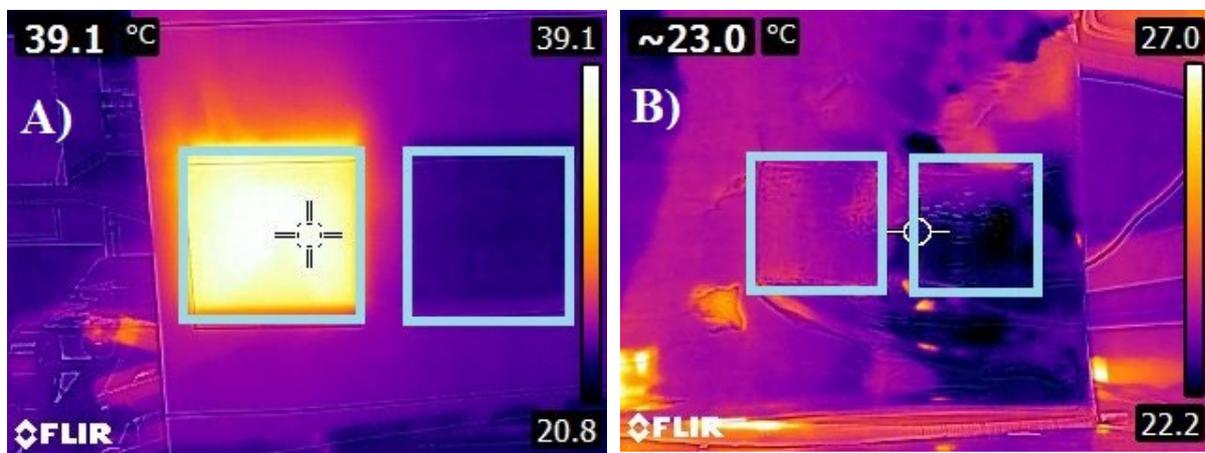


Figure 16 FLIR Infrared picture of the TECs with paper (A) and adhered aluminum foil (B)

Autoscaled false-color thermal infrared images of the TECs (light blue frames) with either paper (A) or aluminum foil (B) glued on. Color scale is shown on the right of each image and the spot temperature in the crosshairs is shown in the upper left. At both pictures is the left side the warm (40°C) and the right side the cold side (22.8 C°, room temperature). A shows the warm paper-covered TEC (left) with measured 39.1°C (crosshairs) and B shows the warm TEC element (left) at 40°C covered with aluminum foil measured at 23 °C (crosshairs, IR camera). The 'bright, orange' areas at the aluminum foil (B) are IR reflections emitted by the surroundings (*e.g.* experimenter).

### 2.2.2 Chemicals

For the mosquito experiments three chemicals (two main insect repellents and one TRP channel activator) were tested in order to check the influence on the mosquitos' heat-seeking behavior.

DEET, N,N-Diethyl-m-toluamide, is a known insect repellent (Table 1) and was developed by the US Army in 1946 to protect military personnel (Jackson 2008). DEET can also exist as a vapor, especially important for the evaporation experiments within the mosquito cage, and degrades via hydroxyl radicals with an estimated half-life of 15 hours (Little 1982). DEET was ordered from Sigma Aldrich and used with 97% purity for all assays. For instance, the accumulation experiment used four times 0.5 $\mu$ L DEET steps (0.000708 ppm), whereas the overnight evaporation was done onetime with of 5 $\mu$ L DEET.

Citronellal, ( $\pm$ )-3,7-Dimethyl-6-octenal, is a component of citronella oil and known as an insect and animal repellent. It is commonly found in candles, lotions, gel, etc. and is known for its efficacy (repellency effect) against mosquitos, biting flies and fleas. In general, the length of repellency time varies with the ingredients and the amount of citronella oil (EPA 1999). For the mosquito experiments 5 $\mu$ L 95% pure Citronellal was vaporized (**Table 1**).

Cinnamaldehyde, 3-Phenylprop-2-enal, is a TRP channel activator and designated by the EPA as a 'New Pesticide Active Ingredient' (a chemical that is not previously registered as pesticide) (EPA 1998). The compound was ordered from Sigma Aldrich and was used once with 5 $\mu$ L in 95% purity (Table 1) for the mosquito experiment.

#### **Table 1 Tested compounds**

	Repellents		TRP channel activator
	DEET	Citronellal	Cinnamaldehyde
CAS Number	134-62-3	106-23-0	104-55-2
Molecular Weight [g/mol]	191.27	154.25	132.16
Boiling point (bp) [°C]	111 (°C/1 mmHg)	206-208	248
Flash Point (FP) [°C]	~ 145-155	75	125 (open cup)
Assay (purity in %)	97	95	95
Supplier	SigmaAldrich	SigmaAldrich	SigmaAldrich

## 2.2.3 Behavioral assays

### 2.2.3.1 Heat-sense with and without IR in Mosquitos

For this experiment, two TEC were used with either paper or aluminum foil glued on (Fig. 14 A). The mosquito cage was tested with at 2, 5 or 10 cm distance from the TECs. One TEC was heated up to 40°C, whereas the other side was set at 22.8 °C. The experiments with the aluminum foil blocked the IR-radiation (IR-reflection plate). The TEC elements were not interchanged, i.e. the assigned hot block was always used as the warm source. To overcome side preference choices and mosquito recovery, the cage was removed from the TECs for five minute breaks between the experiments. To block convective heat transfer, Saran wrap (IR-transparent) was applied at the front facing the TEC plates. Each distance was tested for 5 minutes with approximately 800 mixed-gender mosquitos per cage.

### 2.2.3.2 Heat-sense in Female and Male Mosquitos

The previous experiments included both sexes with an approximate 1:1 female to male ratio. However, this experiment investigated the heat-seeking behavior of both separated male and female mosquitos with the same setup as the experiments above but with 1 cm distance. It is important to note that this experiment was performed at 1 cm distance without Saran Wrap (Fig. 14). Saran Wrap was not needed, as the mosquitos showed from the start a distinct separation between the warm and the cold TEC. Before the experiments, a preselection was done in order to check if the mosquitos in the rearing cage responded to the ‘warm’ stimulus. The sex

separation was done based on the insects morphology (Fig. 18) as females are 2-3 times bigger in body size and do not have feathery structures on the antennae (Fig. 18, red arrow (female) and green arrow (male)). The mosquito sex selection was done with an aspirator (Fig. 17).

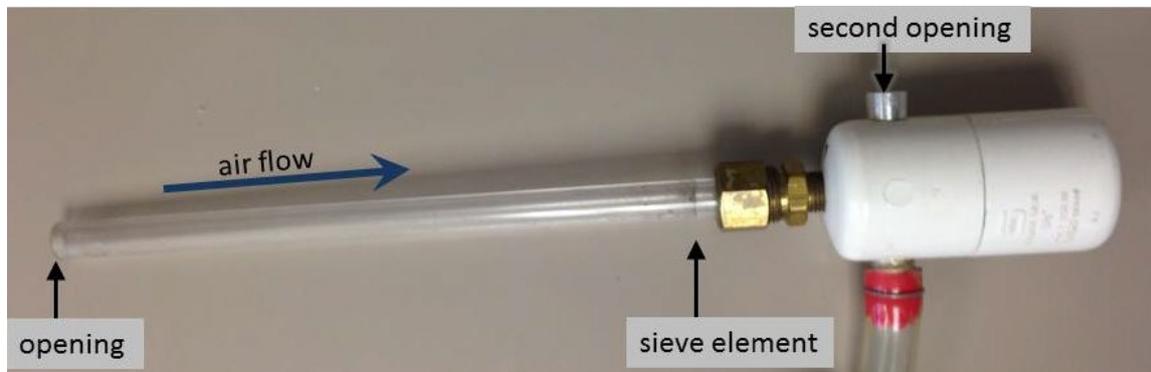


Figure 17 **Mosquito aspirator**

The mosquito aspirator was used to collect the mosquitos out of the rearing cage (Fig. 13). The aspirator collects and transfers insects by positive pressure (airflow out of second opening, against arrow). A build in sieve element holds the insects back in the transparent collecting tube. Blocking the positive pressure with a finger over the 'second opening' relocates the air stream through the collecting tube with the result of blowing the collected mosquitos out. The mosquitos were stored in a box and later anesthetized and selected by gender.

Before the experiment started the mosquitos were temporarily stored in a plastic box awaiting anesthetization by CO<sub>2</sub>. The separation of female and males was quickly performed due to the body size and the antennae morphology (Fig. 18 A vs. B). The mosquitos were grabbed with a light forceps at the hind legs and were put through a funnel into a smaller plastic box with room air for recovery. The recovery time was set to one hour. After that, the plastic container of each sex (n = 107) was released into a clean mosquito rearing cage (Fig. 13) with a 10% cane sugar feeder. The uneven sample size of 107 mosquitos was chosen to have a minimum amount of at least 100 individuals in case any transfer injury occurs. The experiment was performed for 5 minutes with three repetitions.

### 2.2.3.3 Heat-sense in Mosquitos with Amputated Antennae

In order to verify the location of the IR sense organs, the mosquitos' antennae were removed (Fig. 18, C, red bars). Along the current hypothesis are the responsible IR sensing organs, the coeloconic sensillae, located at the antennae as seen for *An. gambiae* (Fig. 19, A-D, red circles) and for *A. aegypti* (Fig. 2, red corcles). The insects were tested as the previous experiments

(Fig. 14 A, top view). A paper sheet (store brand: Staples, Size: 8.5 x 11 inches, weight/sheet: 4.6 g (average of 3 sheets), color: white) was glued on the TECs which were set to 40°C (warm) and 22.8°C (cold). Saran Wrap was also used to cover the front side of the cage facing the TEC to prevent convective heat transfer. The cage with the sample size of 100 mosquitos (per trial – cut or touched antennae) was tested for 5 minutes at 2 cm distance from the TECs.

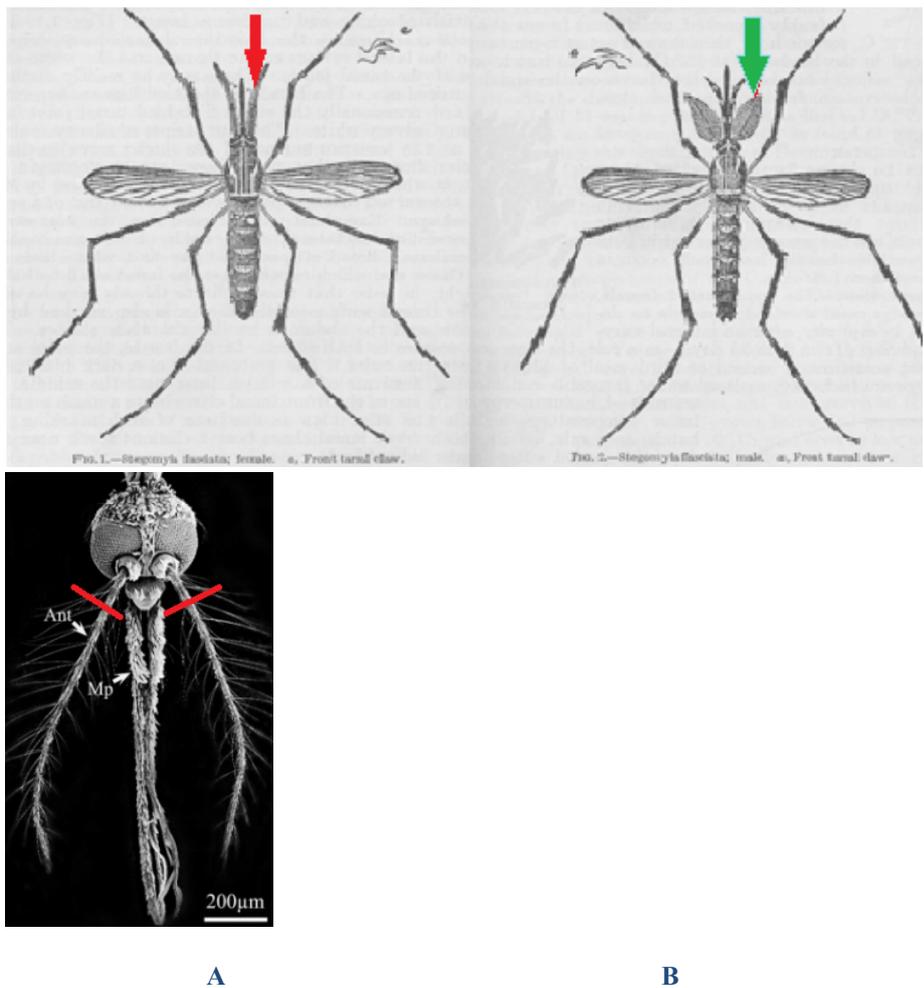


Figure 18 *Aedes aegypti* mosquitos antennae morphology and removal

The female *Aedes aegypti* mosquito antennae (A, red arrow) in comparison to the feathery male mosquito antennae (B, green arrow). The scanning electron microscope image (C) shows an *Aedes aegypti* head with the two red bars indicating the point where the antennae were cut. For instance shows the *Anopheles gambiae* antennae coeloconic sensillae (Fig. 18 A-D, red circles) which are potential thermosensitive organs responsible for IR-seeking and are as well supposed to be found in similar morphology in *A. aegypti* mosquitos. Picture A and B modified from Owen, R.L. (Philadelphia 2009), Picture C modified from Ghaninia and Ignell (Ghaninia, Ignell et al. 2007)

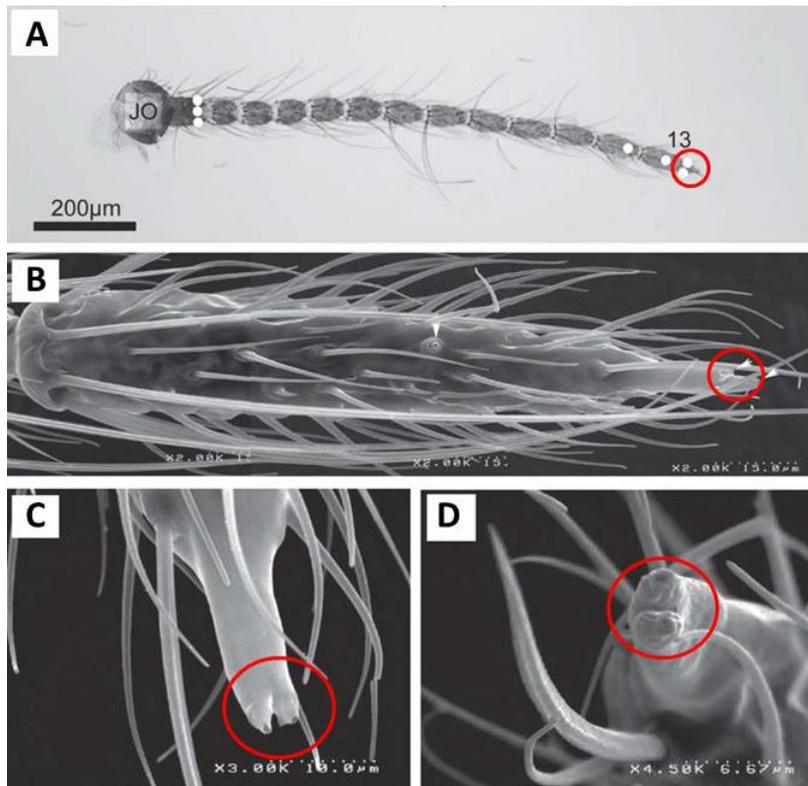


Figure 19 Coeloconic sensillae of *Aedes aegypti*

The graph shows the ultrastructure of the *Anopheles gambiae* antennae with the small coeloconic sensilla (A, white dots). The red circle in B indicates the coeloconic sensilla on the 13<sup>th</sup> antennal segment, whereas C and D show the coeloconic sensilla on the tip of the antennae.

Picture modified from: (Wang, Qiu et al. 2009)

Beforehand, mosquitos were selected out of the rearing cage with an aspirator (Fig. 17) into a temporary plastic container. After the collection, all mosquitos were anesthetized with CO<sub>2</sub> (maximum five minutes per batch (20-25 individuals)) by placing them on a porous, gas-emitting polyethylene surface (FlyStuff Flypad, Genesee Scientific, standard size 8.1cm x 11.6 cm, flystuff.com) to either touch (mock) or cut their antennae (treatment) of both sides. For the ‘treatment’ trial, both antennae were cut close to the mosquito’s head with a dull end left behind to limit the damage (Fig. 18, C, red bars). A complete cut was not necessary as the potential temperature sensitive organs are suggested to be located at the distal tip of the antennae. The antennae amputation was done with a small Vannas scissor (WPI #500260). In the mock the same location at the antennae was touched. Before the mosquitos were tested in the cage (Fig. 13), all handled mosquitos recovered for 2 hours and 15 minutes in a plastic jar. The first results

showed no response from either trail, therefore the recovery time was extended overnight. After 23h recovery time, the mosquitos responded positive by navigating to the heat source. The experiment was performed for 5 minutes with one repetition and 100 female per cage.

#### 2.2.3.4 Heat-sense in Mosquitos with Blocked Convection

The aim of the experiment was to block convection. The experimental setup was done in the same way than all previous experiments (Fig. 14 A) with 2 TECs both at 40°C and 22.8°C. The plastic foil covered the front side of the mosquito cage facing the warm source (see Fig. 14 A, lower graph, green). The used plastic foil was Saran Wrap (SC Johnson, Saran Premium Wrap (low-density polyethylene, LDPE), thickness: 0.6 mm). The plastic foil features high IR transmissivities between 0.95 - 1, therefore, it appears to an IR sensor as an unshielded heat source with the same apparent temperature. Printer paper was glued at the TECs to enhance the contrast for the video recording. The experiment was repeated eight times with 800 mosquitos (mixed gender) per cage.

#### 2.2.3.5 Long-term Heat-sensing of Mosquitos

To test whether *Aedes aegypti* mosquitos have a time-dependent limit for IR sensing, a 12 hour assay was conducted. The TECs were set as usual with 40 °C at the warm and 22.4°C at the cold TEC (Fig. 16 A). The long-term experiment was performed in the mosquito arena (Fig. 14 B) with an open top to prevent an internal air temperature increase. Saran Wrap covered the front side of the cage, which was set 2 cm away from the TECs. The experiment was done overnight from 9 pm to 10am next day with the full laboratory light on. The experiment was performed once with 800 mixed-gender mosquitos per cage.

#### 2.2.3.6 Temperature-dependent Heat-sensing in Mosquitos

This experiment examined the mosquitos' heat-seeking behavior towards different temperatures. The same experimental setup (Fig. 14 A, top view) was used as in all previous experiments with the exception of not covering the cage front with Saran wrap. However, the experiment started out with decreasing temperatures at the warm TEC from 40 °C to 30 °C in 1 °C steps. Throughout the experiment was the cold TEC set at 22.8°C. Additional tested

temperatures at the warm TEC were: 22.4 °C, 25 °C, 45 °C, 50 °C, 60 °C, 70 °C and 80 °C. The same mosquito cage was used repeatedly for all experiments with a five minute break between each temperature change.

#### 2.2.3.7 Heat-sensing and the Effect of the DEET on Mosquitos

Experiments with DEET were performed in the closed arena (see Fig. 14 A) with the warm 40°C and cold 22.8°C TECs. The mosquito's heat-seeking behavior was tested under the vaporized DEET. The accumulative dose-response experiments started with 0.5µL steps (0.000708 ppm) at every 5 to 7 minutes until the total amount of 2µL DEET (0.002125 ppm) was vaporized. In order to evaporate the repellent, DEET was pipetted at a soldering iron (Weller WES51 Soldering Station) and inserted through the wax-covered opening (Fig. 14 A, wax-covered valve). The soldering iron was turned on and a small fan was running for 1 minute to equally distribute the compound throughout the arena. The vaporization started between 20 to 60 seconds after heating up and latest fully evaporated at 1 minute and 30. The mosquitos' behavior was recorded with a standard camera recorder (Bell and Howell DNV16HDZ, Full 1080p HD Infrared Night Vision Camcorder). The accumulative DEET experiment was once repeated with 800 mosquitos per cage.

DEET concentration in the closed arena (ppm = part per million, ppt = parts per trillion)

- 0.5µL DEET/90.09L (0,000708 ppm = 708 ppt (mol/mol))

- 1µL DEET/90.09L (0.001416 ppm = 1416 ppt (mol/mol))

- 1.5µL DEET/90.09L (0,002125 ppm = 2124 ppt (mol/mol))

- 2 µL DEET/90.09L (0,002833 ppm = 2832 ppt (mol/mol))

For all calculations the SATP standard conditions of 101,3 kPa and 25°C was used.

Further, the mosquito behavior under long-time DEET exposure was analyzed. Therefore, the same setup as before was used with 2µL DEET exposure and a test time of 16 hours. DEET was fully vaporized with a soldering iron at the start of the experiment until the mosquitos were inhibited to orient to the warm TEC. Then, the hood was opened and the chamber was well ventilated. The DEET-exposed mosquitos were put back in front of the two TEC elements for 16 hours and were tested for the DEET-recovery.

#### 2.2.3.8 Heat-sensing and the Effect of Citronellal on Mosquitos

In order to study the effects of evaporated Citronellal repellent on the IR-sense organs, the same experimental setup as for DEET was used with the 2 TEC elements (40°C and 22.4°C) in a closed chamber.

At the beginning were also 0.5 µL steps Citronellal evaporated with the soldering iron (Weller WES51 Soldering Station). No effect was observed with 1.5 µL compound, therefore the repellent amount was increased to 5µL (0.007526 ppm). For the experiment, a new cage was used to exclude incorrect doses with direct evaporation of 5µL Citronellal. The evaporated compound was distributed with a small battery-powered fan. The Citronellal experiment was performed once with approximately 800 mosquitos per cage.

Citronellal concentration in the closed arena (ppm = part per million, ppt = parts per trillion)  
- 5 µL Citronellal/90.09L (0,007526 ppm = 7526 ppt (mol/mol)

For all calculations the SATP standard conditions of 101,3 kPa and 25°C was used.

#### 2.2.3.9 Heat-sensing and the Effect of Cinnamaldehyde on Mosquitos

To determine the role of cinnamaldehyde on the mosquitos heat-seeking behavior, the same experimental setup with the closed arena was used (Fig. 14 B) as described for DEET and citronellal. As usual, the TECs were kept at 40 °C (warm side) and at 22.8°C (cold side).

5µL Cinnamaldehyde (0.001079 ppm) was directly applied to the soldering iron and inserted through the wax-covered valve (Fig. 14 B, arrow) until the compound was fully vaporized at 2 minutes and 30 seconds. The evaporated compound was distributed with a small battery-powered fan. After the recording, the chamber was ventilated with a bigger fan for 15 minutes before the cinnamaldehyde recovery experiment started. The recovery of the tested mosquito cage was done with room air (same lab). For the recovery experiments were the TECs set at 40 °C (warm side) and at 22.8°C (cold side) with an open arena. The Cinnamaldehyde experiment was performed once with 800 mosquitos per cage.

Cinnamaldehyde concentration in the closed arena (ppm = part per million, ppt = parts per trillion):

- 5 µL Cinnamaldehyde/90.09L (0,001079 ppm = 1079 ppt (mol/mol)

For all calculations the SATP standard conditions of 101,3 kPa and 25°C was used.

## 2.2.4 Video and Data Analysis

The HD-videos were converted to JPGs (file format: Joint Photographic Experts Group) with the 'Free Video to JPG Converter' program (Free Video to JPEG Converter, DVDVideoSoft, Digital Wave Ltd.). The conversion into the jpeg format was done to reduce the size and process the picture stacks (Fig. 20) them with ImageJ (US National Institutes of Health, Bethesda, Maryland, USA). All stacks were cropped to show only the two TECs individually to reduce the processing time. For further processing, a self-written ImageJ plugin (see below) was used.

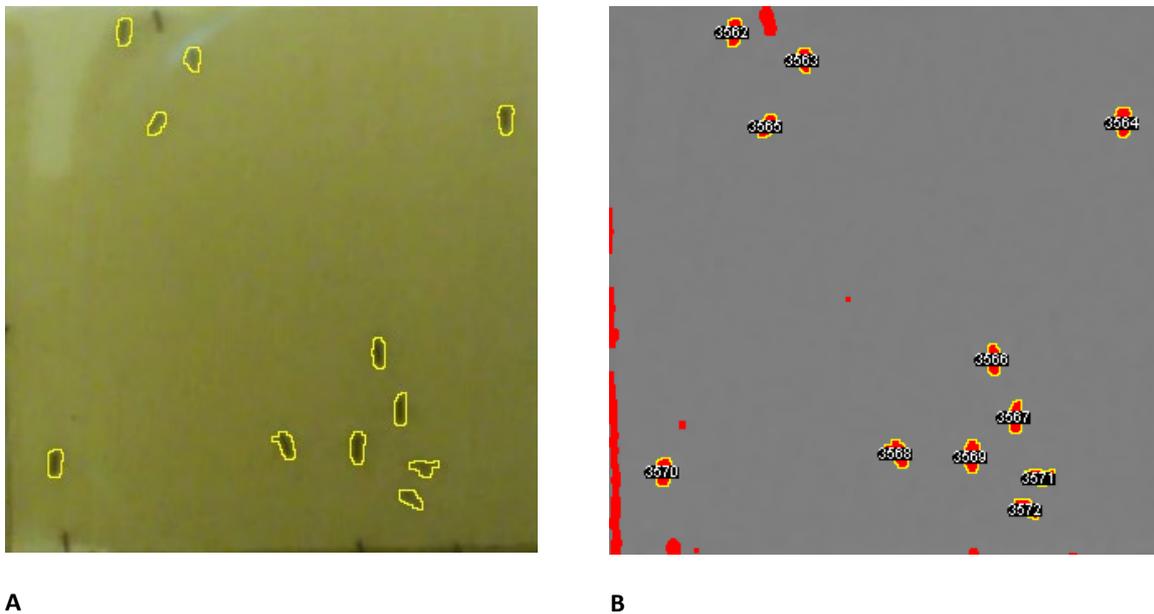


Figure 20 **Overview of the mosquito counting process with ImageJ**

The left picture (A) depicts the 'automated', self-written mosquito counting plugin in ImageJ. The program was developed to circle mosquito shaped objects with yellow lines (A). This image stack was further processed (B) with a uniform background to enhance the contrast and mark the darker objects with red color. This method was robust to count the individual mosquitos over time at the TECs.

### ImageJ code to process the cropped picture stacks for mosquito counting:

```
rename("Original");  
run("Duplicate...", "title=Duplicate duplicate stack");  
run("HSB Stack");  
run("Split Channels");  
selectWindow("C1-Duplicate");  
run("Close");
```

```

selectWindow("C2-Duplicate");
run("Close");
selectWindow("C3-Duplicate");
run("Unsharp Mask...", "radius=15 mask=0.90 stack");
run("Bandpass Filter...", "filter_large=400 filter_small=1 suppress=None tolerance=5
autoscale saturate process");
run("Gaussian Blur...", "sigma=3 stack");
run("Convolve...", "text1=[-1 -1 -1 -1 -1\n-1 -1 -1 -1 -1\n-1 -1 25 -1 -1\n-1 -1 -1 -1 -1\n-1 -1 -1
-1 -1\n] normalize stack");
setAutoThreshold ("Minimum");

```

ImageJ code to count the mosquitos in the picture stack:

```

run("Analyze Particles...", "size=50-400 circularity=0.00-1.00 show=[Overlay Outlines]
exclude clear summarize add stack");

```

### 2.2.5 Graphs and Statistical analysis

All the output of the results were saved as Microsoft Excel files, in which the values for every 30 seconds (1 frame = 1 second) have been marked. Another table was created of all 30 second values of mosquito numbers at the warm and cold TEC over time. All mosquitos' graphs were plotted with the mean numbers and the standard error of the mean with Prism 6 (Graph Pad Software, USA).

### 3. Results

#### Results mosquitos

##### 3.1 Heat-sense in Mosquitos at different distances

Several assays (see assay protocol) were performed to test whether *Aedes aegypti* mosquitos sense and navigate to a warm IR-emitting source.

Figure 21 shows the number of mosquitos that oriented to either a 40 °C warm source (red circles) or a 22.8 °C cold source (blue squares) over a time span of five minutes. At the start, a difference in navigation between the two TECs was noticed as 13 mosquitos oriented to the warm side, whereas 9 individuals were counted at the cold source. After 150 seconds, 25 individuals oriented to the warm side, whereas 10 mosquitos were found at the cold source. The attraction to the warm gradually increased towards the end of the test. At 300 seconds, 30 Mosquitos oriented to the warm side, whereas were 8 individuals found at the cold source (Fig. 21 and Fig. 26). The difference between the warm and the cold source is significantly different (Student's t-test,  $p=0.0002$ ). Overall, the greater number of mosquitos orienting towards the warm source showed that they potentially used IR to detect an IR-emitting source from 2 cm distance. Further experiments with 5 and 10 cm distances were done to analyze whether mosquitos can also orient to IR-emitting sources at greater distances.

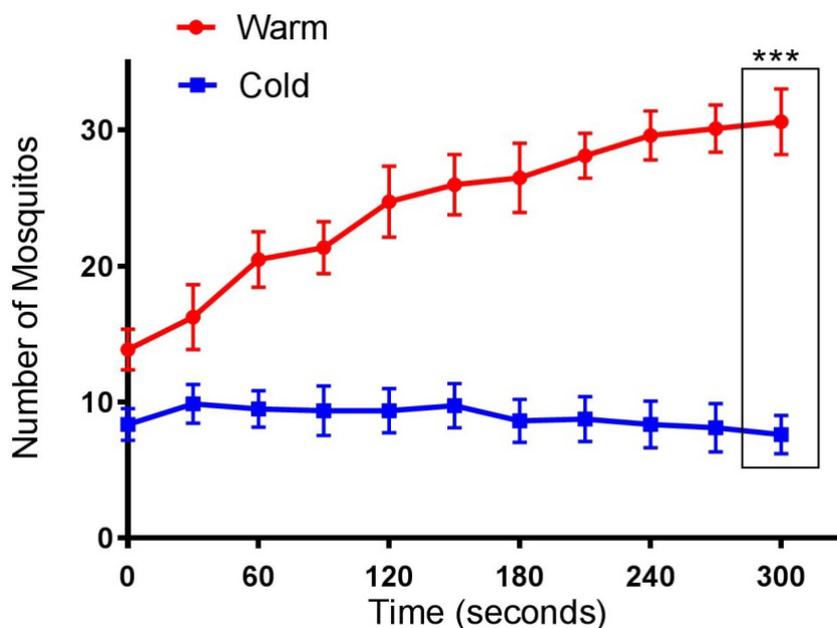


Figure 21 Mosquito navigation towards an IR-emitting warm plate at 2 cm distance

TECs were held at 40 °C (warm) and 22.8 °C (cold). The red line (circles) indicates the number of mosquitos navigating to the warm side whereas the blue line (squares) shows the number of mosquitos at the cold side. From the start, a difference in number of individuals navigating to either the cold and warm TECs occurred. In numbers, 13 mosquitos oriented to the warm side and 9 individuals to the cold side. At the end of the experiment (300 seconds) navigated 30 mosquitos to the warm side whereas 8 mosquitos to the cold source. Throughout the entire experiment increased the number of mosquitos at the warm side from 13 to 30. In comparison, the number of mosquitos at the cold TEC stayed between 8-10 individuals. The number of mosquitos at 300 seconds between the warm and cold is significant different (Student's t-test,  $p=0.0002$ ). The cage used for this experiment contained around 800 mosquitos and was repeated eight times.

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The 5 cm distance experiment (Fig. 22) shows no difference between the numbers of mosquitos that oriented to either the warm or the cold side until 30 seconds. After 30 seconds, more and more mosquitos oriented to the warm, IR-emitting source (Fig. 22) with an increasing trend towards the end of the experiment. In the middle of the experiment (150 seconds), 12 mosquitos navigated to the warm source whereas 8 individuals were found at the cold side. The maximal difference of 7 mosquitos can be found at 300 seconds between the warm (15 mosquitos) and cold source (8 mosquitos) (Fig. 22 and Fig. 26). However, the number of mosquitos between the warm and cold side is significant different (Student's t-test,  $p=0.0193$ ), therefore it can be said that the mosquitos are able to navigate to an IR-emitting source at 5 cm distance.

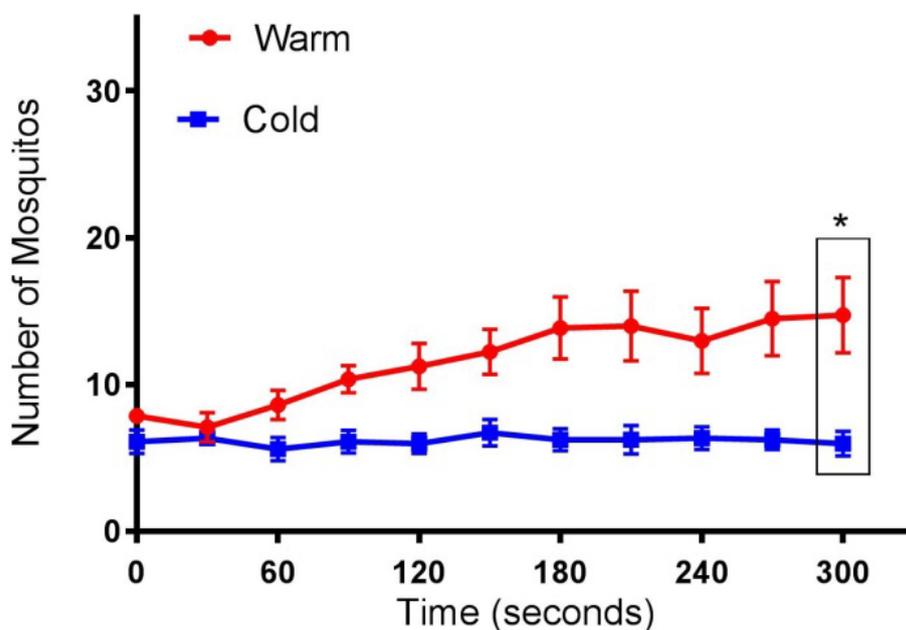


Figure 22 Mosquito navigation towards an IR-emitting warm plate at 5 cm distance

This graph shows similar results as figure 21 (at 2 cm distance) with a choice between 40 °C (warm) and 22.8 °C (cold). The red line (circles) depicts the orientation to the hot side, whereas the blue (squares) the orientation to the cold one. No difference until 30 seconds was observed between the number of mosquitos at the warm and cold side. After 30 seconds, the number of individuals at the warm side increased, whereas the cold side stayed constant with around 8 individuals. At 60 seconds 10 mosquitos oriented to the warm side and at 180 seconds around 15 which remained constant until the end (300 seconds). At 300 seconds (5 minutes), there is a significant difference (Student's t-test,  $p=0.0193$ ) in the number of mosquitos between the warm (10 mosquitos) and the cold source (9 mosquitos). The used cage contained around 800 mosquitos and the experiments were repeated eight times (same cage).

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Figure 23 shows another distance experiment at 10 cm with mosquitos orienting to either the warm or cold side (Fig. 23). The data shows no difference in the numbers of mosquitos (ranges between 5 to 8 individuals both sides) between the warm and the cold TEC until 150 seconds. After 150 seconds, 13 individuals navigated to the warm source whereas 8 stayed at the cold side. This trend remained constant until the end of the experiment at 300 seconds. At 300 seconds, 12 mosquitos oriented to the warm side and approximately 8 to the cold side. No statistic was done as the sample size was too low (2 values). However, a 3<sup>rd</sup> added value at 300 seconds would have made a t-test possible, i.e. adding a 3<sup>rd</sup> averaged value (data not shown) will show a significant difference between the warm and the cold source. Overall, the results still show a difference of 4 individuals between the warm and the cold side, therefore mosquitos are still able to navigate to the warm, IR-emitting source at 10 cm.

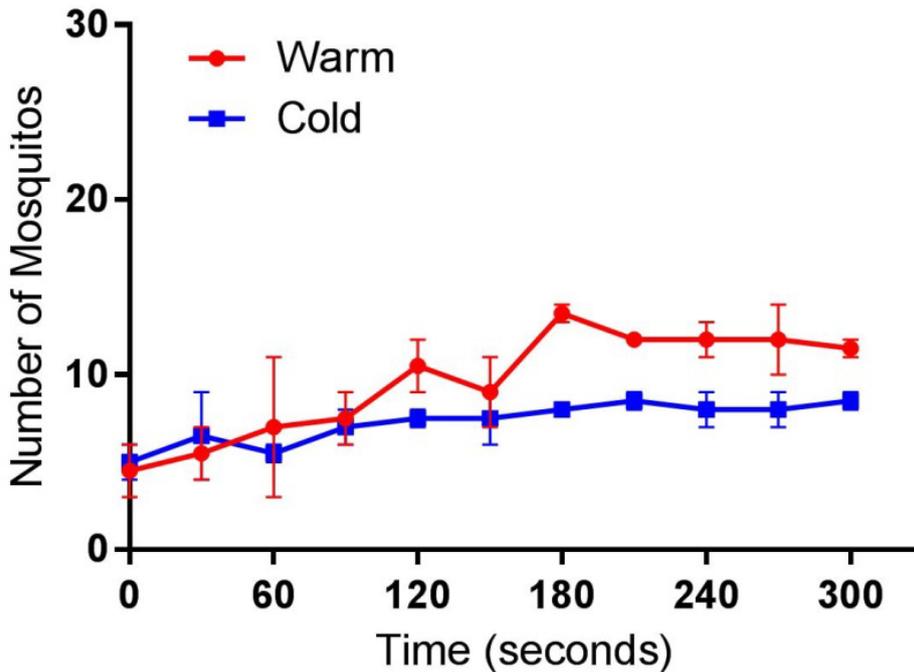


Figure 23 Mosquito navigation towards an IR-emitting warm plate at 10 cm distance

In the 10 cm distance experiment, the mosquitos also had the choice between 40 °C (warm, red) and 22.8 °C (cold, blue) TEC plates. From the start of the experiment there has been no difference between the numbers of mosquitos at either side. At 150 seconds, 13 mosquitos navigated to the warm side whereas 8 stayed at the cold side. This trend remained constant until the end (300 seconds) of the experiment with 12 mosquitos at the warm and 9 at the cold side. No statistic was done for the 300 time point due to the low sample size of 2. The experiment was repeated two times with approximately 800 mosquitos in the cage

### 3.2 Heat-sensing in Mosquitos with non-IR-emitting warm plate

Mosquitos are able to sense infrared radiation, which has been shown by the previous results. The next step was to check the mosquito's heat-seeking behavior with a warm, IR-emission blocked source. Therefore, aluminum foil was used to block the IR radiation of both TEC plates (Fig. 24). Figure 24 shows the number of mosquitos that oriented to either the IR-blocked 40 °C warm source (red) or the 22.8 °C cold source (blue) over a time course of five minutes. Throughout the entire time span of 300 seconds no difference in the number of mosquitos between the TECs could be seen. At 300 seconds, there wasn't any significant difference (Student's t-test,  $p= 0.4316$ ) among the number of mosquitos between the warm and the cold side. In details, the number of mosquitos at the warm side is even lower than the amount at the cold side. This result shows that the mosquitos were not able to discriminate the warm side at a 2 cm distance when IR-radiation was blocked. These experiments can

further be compared to the ratio experiments in Fig. 25 as the ratio stayed at 1 (or even below), which means that the number of mosquitos between the warm and cold side does not differ.

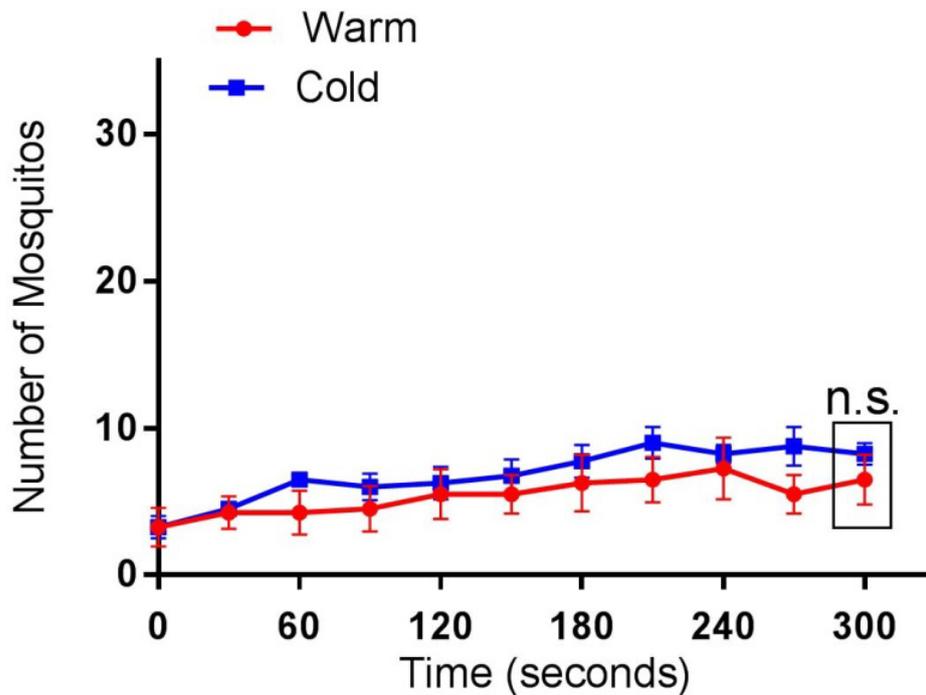


Figure 24 **Mosquito navigation towards an non-IR-emitting warm plate at 2 cm distance**

The figure shows the experiment with the two TECs kept at 40°C (warm, red) and 22.8°C (cold, blue). The number of individuals navigating to the warm side did not differ throughout the entire time span of 300 seconds. At 300 seconds, no significant difference (Student's t-test,  $p=0.4316$ ) in the number of mosquitos between the warm and the cold side was observed. Therefore, the results depict that the mosquito population at each side is similar, *i.e.* around 6-8 individuals per TEC. The experiment was repeated four times (same cage) with approximately 800 mosquitos per cage.

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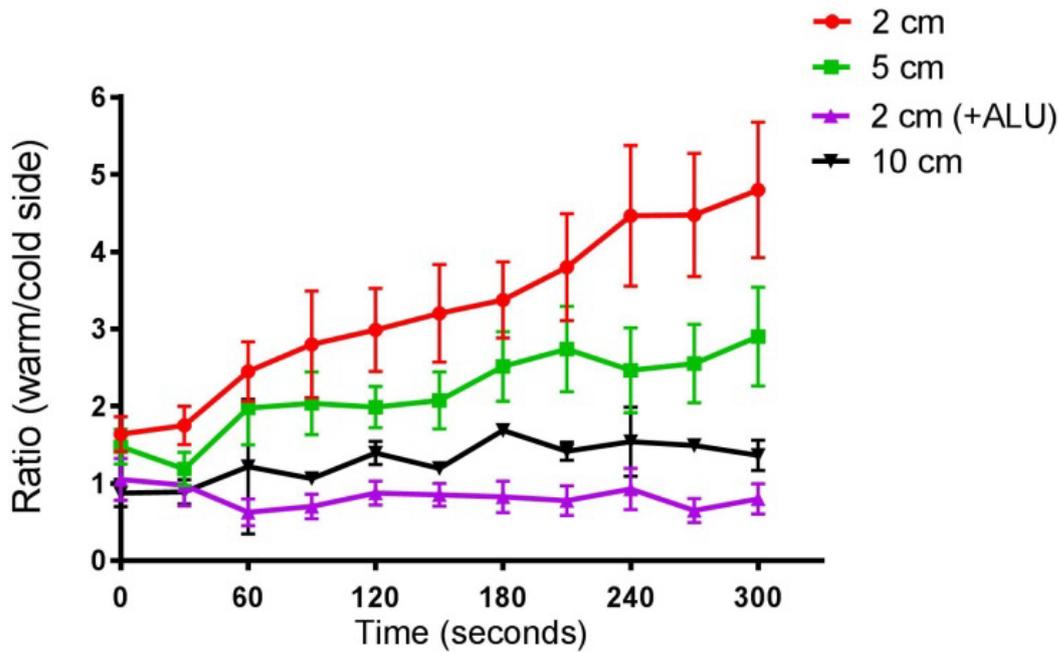
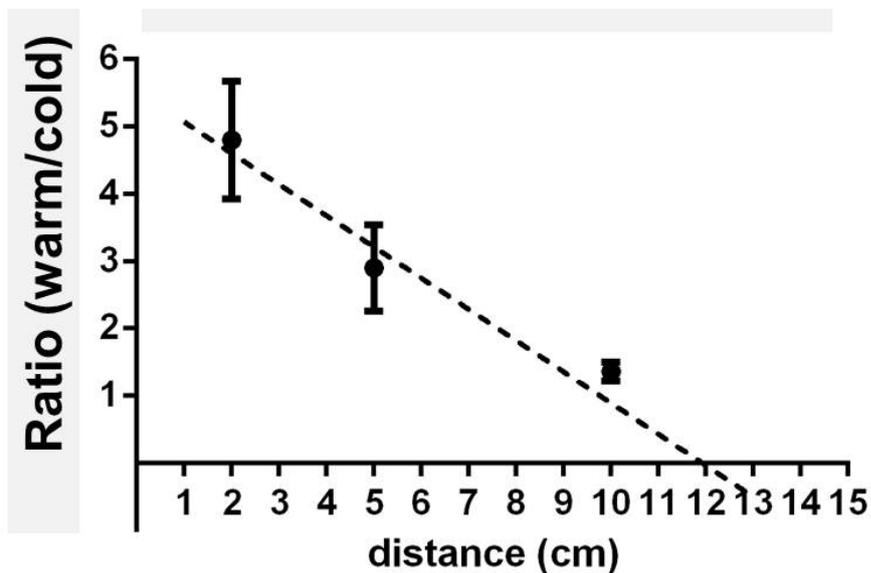
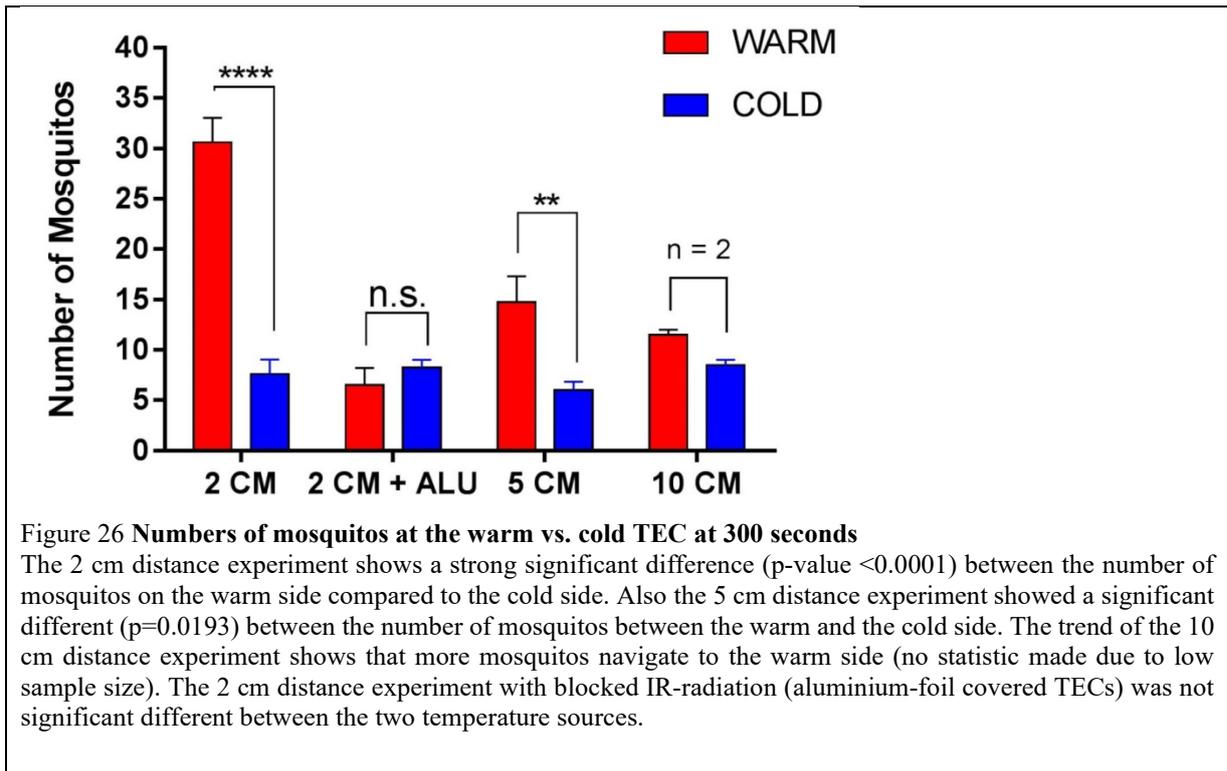


Figure 25 **Ratio of the mosquitos choice tests for the four different experiments in *Aedes aegypti***

The ratio of the shortest distance (2 cm from the cage, red line, circles) steadily increases over time. This means that the number of mosquitos navigating to the warm side increased over time. The ratio at 300 seconds is four times higher than the ‘non-IR-emitting experiment’ (2 cm distance plus aluminum). The ratio for the 5 cm experiment is two times higher than the ‘non-IR-emitting experiment’; therefore mosquitos still navigate to the warm source. The ratio for the 10 cm experiment (black, triangles) shows the smallest ratio at 1.5. That means that the mosquitos are still able to differentiate between the warm and cold source (compared to the 2 cm IR-blocked experiment (purple)). The 2 cm distance experiment with the aluminum foil-covered TEC’s (purple graph, triangles) depicts the ratio of 1, *i.e.* no difference between the orientations to either the warm or cold side was observed.

The summary of number of mosquitos at 300 seconds of the four experiments is shown in Fig. 26. The shortest distance, the 2 cm distance experiment, showed a strong significant difference ( $p$ -value:  $< 0.0001$ ) between the numbers of mosquitos navigating to the warm or cold source. The 5 cm distance experiment results are similar to the 2 cm distance experiment but with a low  $p$ -value of 0.0193. The same trend was seen for 10 cm distance experiment (no statistic made due low sample size). In contrast stands the 2 cm experiment with aluminum foil-covered TECs which shows no significant difference in number of attracted mosquitos between the TEC modules (details see Fig. 24). This concludes that the mosquitos were not attracted to the warm side with blocked IR-emission. An extrapolation of the 3 tested distances (Fig. 27) shows that more mosquitos navigate to the IR source when they are closer. Vice versa, the further away the TECs are, the lower the amount of mosquitos finding the 10 X 10 cm source. The detection limit for the 100cm<sup>2</sup> warm TEC source was determined as 10 cm by extrapolation of the three different distance experiments. The added linear regression line ( $Y = -0.4642 \cdot X + 5.533$ ) helped to estimate the maximum distance of mosquitos navigating to an IR-emitting source (10 X 10 cm).



### 3.3 Heat-sensing in Mosquitos with blocked convection

This experiment tested whether *A. aegypti* mosquitos sense IR differently through the tested transparent plastic foil (Saran wrap). Preliminary experiments were able to show that mosquitos are attracted to objects at 40 degrees at 2 and 5 cm distance. Blocking IR-radiation by covering the warm surface with aluminum foil prevented the mosquitos from navigating to the warm source, showing that the mosquitos were sensing the warm source by IR radiation. As another control to confirm that they were using IR-sensing, we eliminated air convection as a means of sensing the warm TEC by placing transparent plastic foil between the cage and the TEC plates (see Materials and Methods, Fig. 14 A). Figure 28 shows the experiment with the Saran wrap covering the cage front with the two TECs at either 40°C or 22.8°C. At the start, no difference in the amount of mosquitos between the TECs was observed. The individuals at the warm side increased from 10 (start) to 90 mosquitos, whereas the numbers at the cold side constantly had 10 individuals. At 300 seconds, the 90 individuals were attracted to the warm side, whereas 10 individuals were found at the cold side. However, the plastic foil experiment showed good IR transmission as many mosquitos were attracted to the warm source.

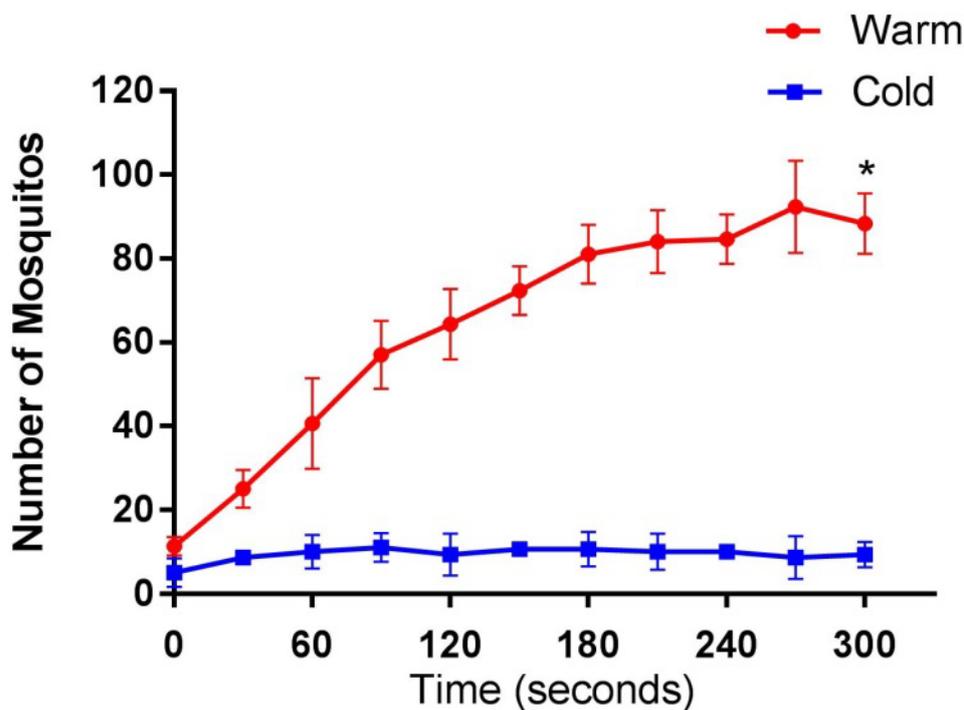


Figure 28 Mosquito navigation towards an IR-emitting warm plate at 2 cm distance with Saran wrap

The figure shows the number of mosquitos navigating to either the warm or cold TEC with the cage covered by Saran Wrap (emissivity: 0.95-1). After the start, the number of mosquitos at the warm side started to increase, whereas the numbers at the cold side stayed constant at 9 individuals throughout the 300 seconds experiment. At 60 seconds, 40 mosquitos navigated to the warm side (approx. 9 at the cold side). The numbers of mosquitos increased until 300 seconds, whereas the amount of mosquitos is significant different (Student's t'-test, p-value: 0.0117) between the warm and the cold side. The experiment was repeated seven times with the same cage.

### 3.4 Heat-sensing in Female and Male Mosquitos

The results so far have shown that Mosquitos orient to an IR source. However, the question of gender difference between the warm and the cold TEC remains unknown. As mentioned in the introduction, female mosquitos are known to suck blood in order to develop the eggs, whereas males feed exclusively on nectar. In the following experiments 107 male and 107 female mosquitos were separately tested for their behavioral response to a thermal source. Figure 29 A shows the number of female mosquitos that oriented to either a 40 °C warm (red) or a 22.8 °C cold source (blue) over a time span of five minutes. The distance between the warm TEC and the cage was 1 cm which was sufficient as the female mosquitos showed a distinct behavior to the warm TEC. Overall, the navigation to the warm source increases over time. At the start of the experiment, 1 mosquito was detected at the warm side but within 150 seconds the number of mosquitos increased to 7 individuals. At 300 seconds, the number of mosquitos increased again to 10 mosquitos. Throughout the experiment only 1 mosquito oriented over time to the cold side. Therefore, the cold side is the 'indirect control' as it was set to room temperature and symbolizes the general surroundings. However, at 300 seconds, the number of female mosquitos navigating to the warm compared to the cold (Fig. 29 C) were significantly different (Student's t-test, p-value: 0.0462). In comparison to the females, the male mosquito experiment (Fig. 29 B) depicts no difference in numbers of mosquitos between the warm and cold TEC over the entire time span. The number of mosquitos stayed low with 1-2 individuals. At 300 seconds (Fig. 29 C), no significant difference between the two TECs could be found. In comparison increased the number of female mosquitos navigating to the warm source, whereas the number of male mosquitos stayed low (1 mosquito at warm side). Therefore, the experiments show that only female mosquitos are attracted to a warm, IR-emitting source, whereas the males are not.

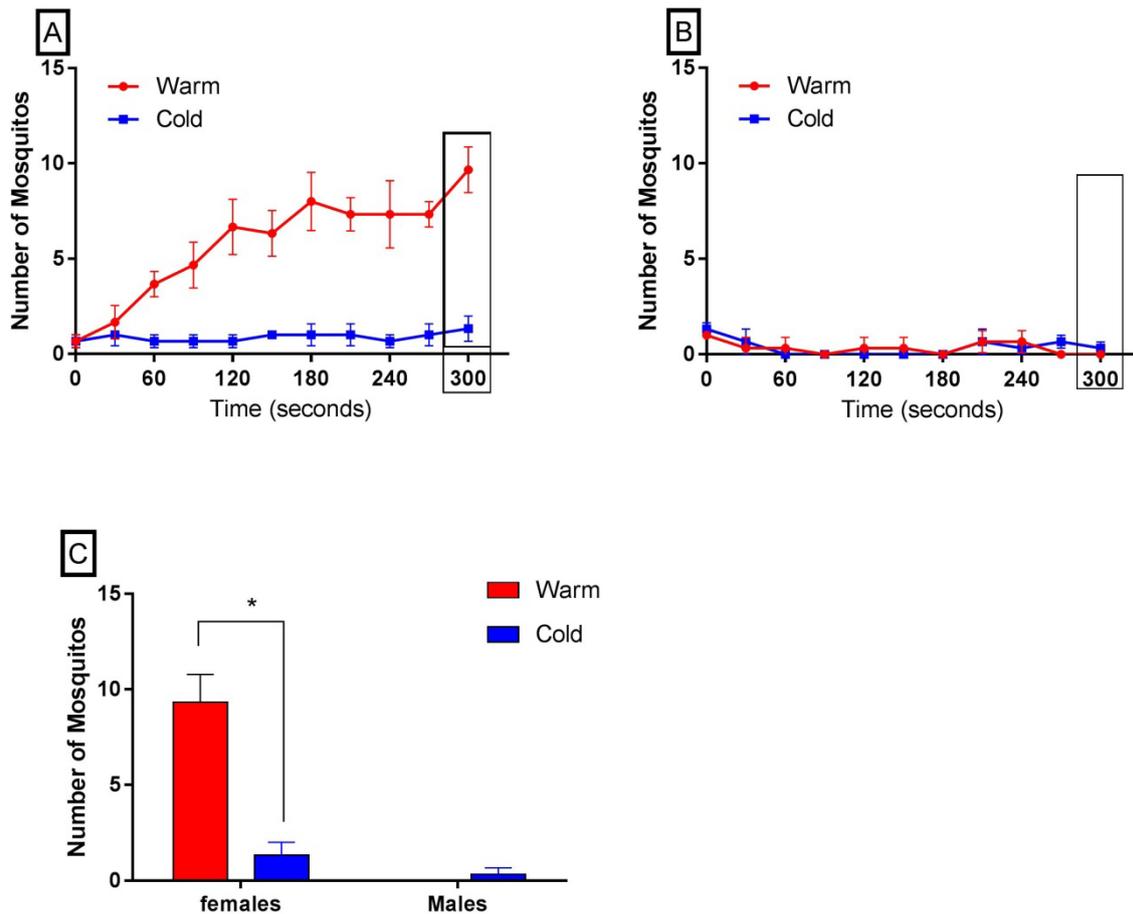


Figure 29 Sex difference of IR-sense behavior in *Aedes aegypti* mosquitoes

A depicts the number of **female mosquitoes** over time orienting to either the warm (red) or cold (blue) TEC. The number of mosquitoes at the warm TEC increased over time from 2 at the start to 7 individuals at 150 seconds and finally 10 mosquitoes at 300 seconds. The number of mosquitoes going to the cold side stayed low between 1 to 2 individuals. Overall, the number of mosquitoes between the warm and cold TEC was significantly different (Student's t-test, p-value: 0.0462; see A and C). B shows the same setup with **male mosquitoes** but there was no difference between the warm and cold side. The bar chart (C) depicts the comparison between the number of mosquitoes and the gender (female/male) at the 300 second time point. The number of female mosquitoes is significant different between the warm and the cold side (Student's t-test, p-value: 0.0462). For the males, no individual preferred the warm side or the cold side (1 mosquito at cold side). This results show that there is no significant difference in the mosquito numbers between the warm and cold TEC within the males (student's t-test, p-value: 0.4226). The experiment was repeated three times with 107 mosquitoes per cage for each sex.

### 3.5 Heat-sense in Mosquitos with Amputated Antennae

The previous experiments have shown that mosquitos navigate to an IR-emitting source. However, the location of the potential IR-sense organ still remained unknown. To test whether *A. aegypti* mosquitos use their antennae to sense IR, two behavioral trials (warm vs. cold TECs) with cut and mock-operated antennae were performed. The first experiment (Fig. 30) shows the

number of mosquitos with cut antennae (Fig. 18, Materials and Methods) that oriented to either a 40 °C warm source (red) or a 22.8 °C cold source (blue) over a time span of five minutes. The results depict no significant difference between the numbers of mosquitos between the warm and cold TEC. This result shows that the mosquitos' antennae might be responsible for the IR-seeking behavior (sample size cut antennae = 76 mosquitos).

The second part of the experiment tested mosquitos with touched antennae at the same location where the antennae were cut off. From the start, no difference between the number of individuals between the warm and cold side was observed. Differences between the warm and cold side were seen from 30 seconds onwards. At 150 seconds, 11 mosquitos oriented to the warm side, whereas no mosquito was found at the cold side. The orientation to the warm side gradually increased at 300 seconds to 15 mosquitos. Overall, the data shows that the mock experiment (touched antennae) has a high significance between mosquitos orienting to the warm and cold source (Students t-test, p-value: <0.0001). This result shows that the mosquitos with touched antennae were not impaired in their navigation to a warm, IR-emitting source. The mosquito cage for the mock treatment contained 79 individuals. The comparison of the number of mosquitos on the warm side between the mock (touched antennae) and the treatment (cut antennae) is also strong significant different (Students t-test, p-value: <0.0001). However, this shows as well that mosquitos need the antennae to detect a warm IR-emitting source.

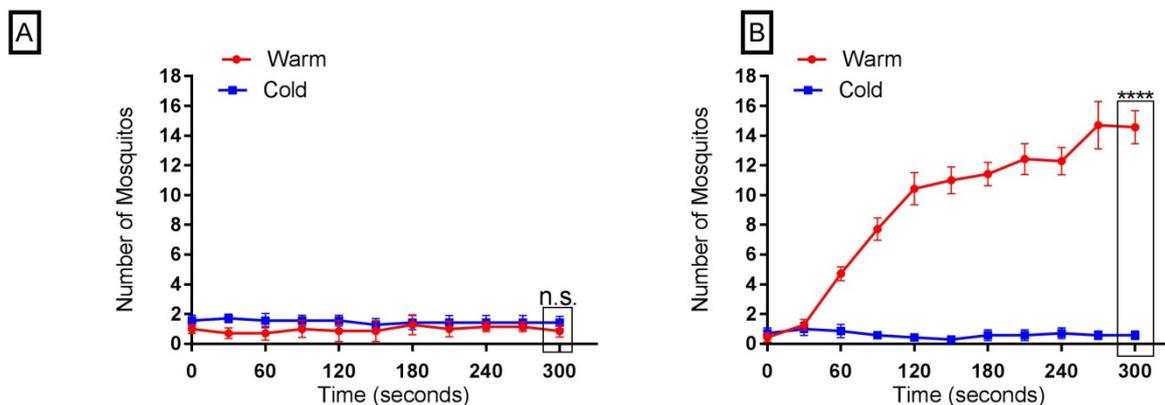


Figure 30 Mosquito navigation towards to a non-IR-emitting warm plate with cut (A) and touched (B) antennae

Figure (A) shows the mosquitos with cut antennae with no difference between the warm and cold TEC throughout the tested 300 seconds. The 300 second time point shows no significant difference between the number of mosquitos between both TECs (Student's t-test, p-value: 0.2308). From the start, the cage contains 100 mosquitos but after the recovery for 23h only 76 mosquitos survived (with sugar feeder). The experiment was repeated 8 times with the same cage. **B** shows the mock treatment, i.e. the mosquitos with the touched antennae as a control. After 300 seconds, there is a significant difference between the warm and cold side of 15 mosquitos (Students t-test, p-value: <0.0001). However, the trend of differences in the navigation behavior between the warm and cold TEC was seen from the start. After 30 seconds was approximately 1 mosquito found

at each side but until 150 seconds increased the orientation towards the warm side. At 150 seconds, 11 mosquitos were found at the warm side, whereas no individual at the cold side. The maximum of 14 mosquitos was reached at 300 seconds. The experiment was started with 100 mosquitos but after the recovery (23h) 79 mosquitos survived. The experiment was repeated 8 times with the same cage.

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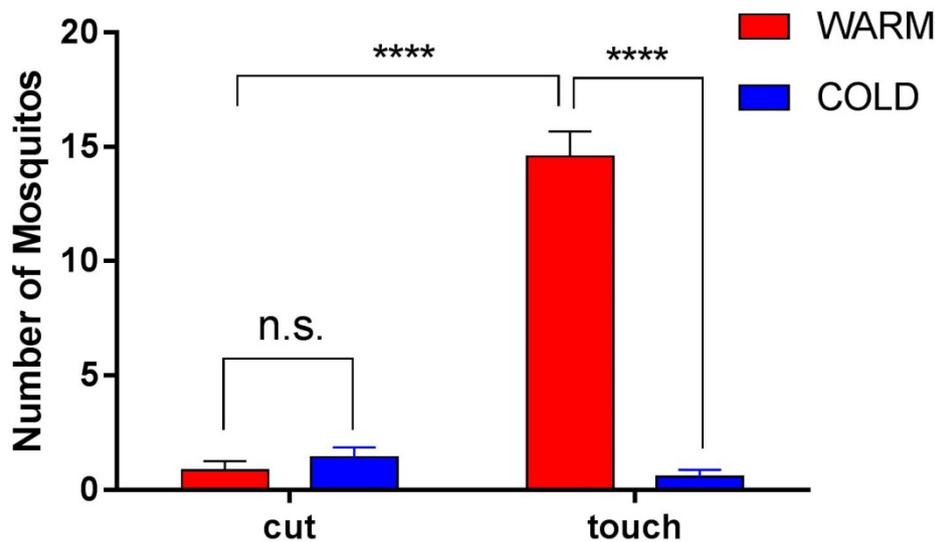


Figure 31 Summary of *Aedes aegypti* heat recognition with cut and touched antennae

The figure shows the mosquito aggregation at the 5 minute time point. There is no significant difference of the mosquitos with cut antennae between the warm and the cold TEC. In comparison stands the experiment with touched mosquito antennae with a significant difference (Students t-test, p-value: <0.0001) between the number of mosquitos navigating to the warm and the cold side. The comparison of the number of mosquitos on the warm side between the mock (touched antennae) and the treatment (cut antennae) is highly significant different (Students t-test, p-value: <0.0001). The total amount of mosquitos for the experiment started out with 100 but ended up with 79 for mock and 76 individuals for the treatment. (\*\*\*\* = p-value < 0.0001)

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### 3.6 Temperature-dependent Heat-sensing in Mosquitos

To test whether mosquitos have a particular temperature-dependent IR sense, an experimental setup was done to test 1 °C steps within the range from 30 to 40 °C. The second part of the experiment also tested temperatures above 40°C in 10°C steps until 80°C. Figure 32 A shows the number of mosquitos at different temperatures starting from 22.4°C, 30°C, 32°C and in 2°C steps to 40°C. Above 40°C were two more ‘extreme’ temperatures with 60°C and 80°C tested. The entire graph shows that the mosquitos’ attraction to the warm source gradually increases with temperature. However, setting the TEC to 40°C (close to human body temperature) looks like the optimal temperature as at 60 seconds almost 30 individuals navigated to the warm TEC.

The number of mosquitos increased to 45 individuals until the end of the experiment at 300 seconds. Crucial for IR-sensing seems to be the interval between 34 to 36°C. The number of mosquitos navigating to the 34°C warm side stayed at approximately 10 individuals. In contrast stands 36°C with 10 mosquitos at the start and increased over time to 20 individuals at 120 seconds. At 300 seconds oriented 30 mosquitos to the 36°C warm side. However, the difference between 34°C and 36°C is 20 mosquitos; therefore might this temperature interval be crucial for the mosquitos to detect a warm IR-emitting source. Figure 32 B shows the number of mosquitos orienting to the warm TEC at the 5 minute point (300 seconds) with a more detailed temperature range from 22.4°C (ambient temperature, bold blue) to 40°C (used experimental temperature, bold red) until 80°C. The results are the same but showing the 1°C steps (than 2°C steps in Fig. 32 A). Here, also 40°C shows the highest number with 45 mosquitos navigating towards the warm IR-emitting source. However, the results show that setting the warm TEC at 40°C attracted the highest amount of mosquitos which was therefore set as the standard temperature for the warm side.

### IR navigation temperature-dependence of *Aedes aegypti*

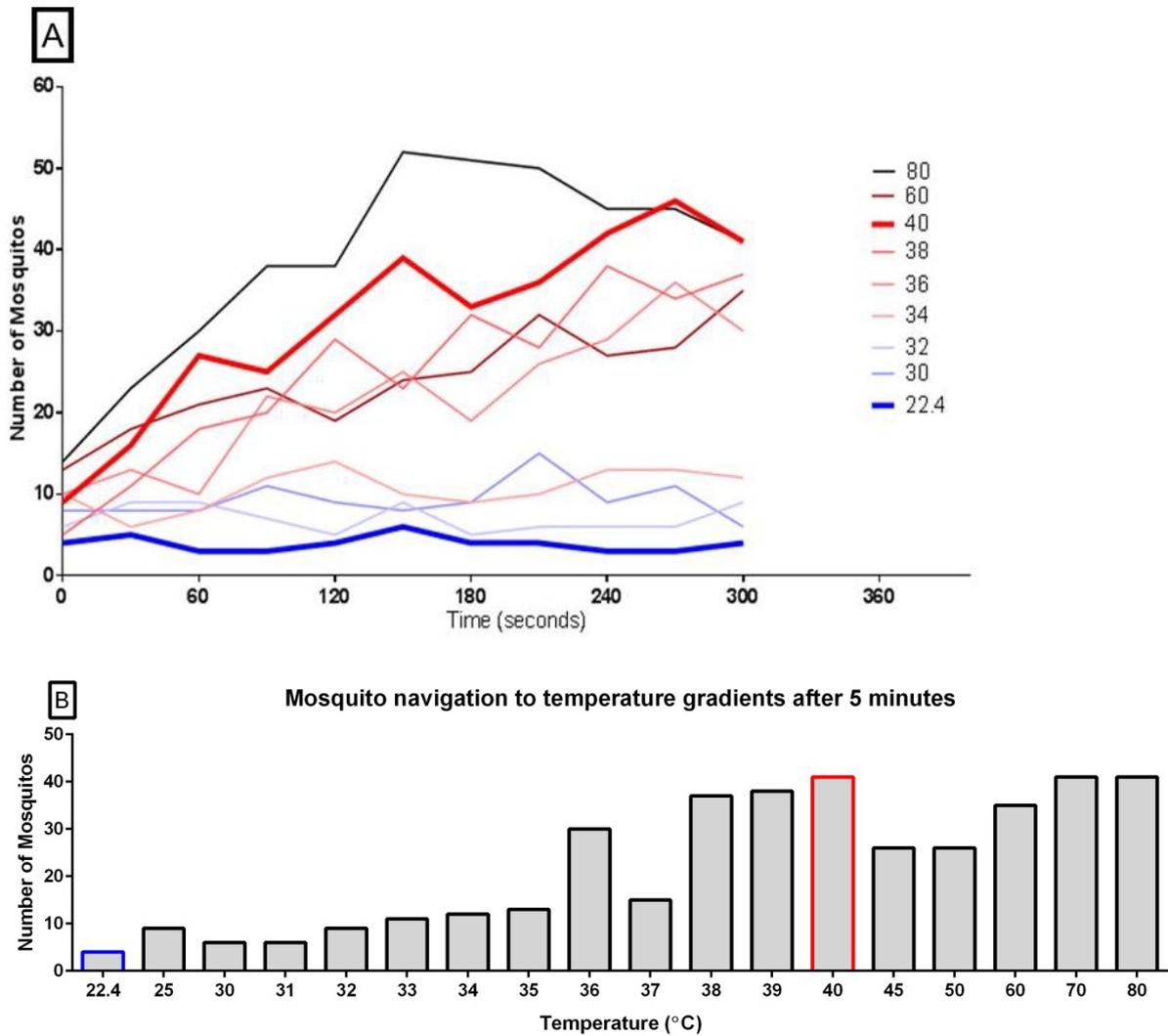


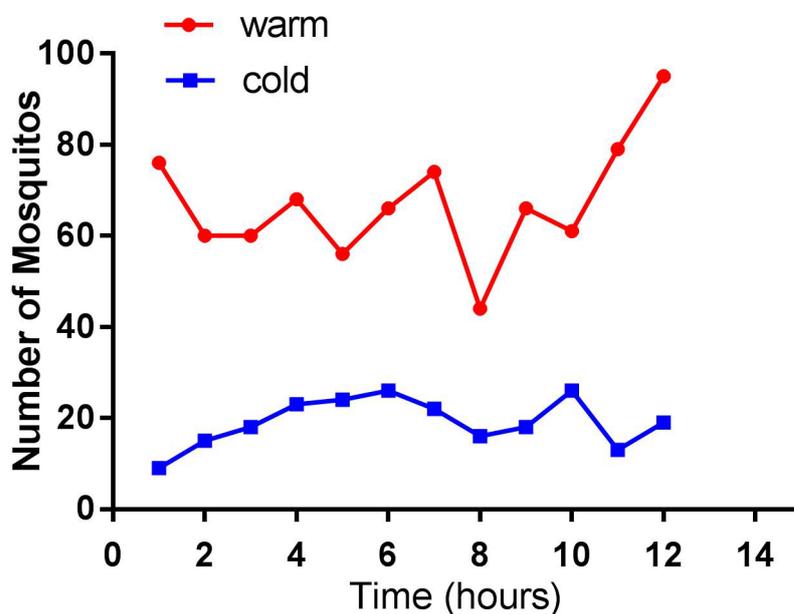
Figure 32 IR temperature-dependent navigation of Adult mixed mosquitos

**A** shows an outline of the experiments with mosquitos orienting to the tested temperatures (every 2 degrees were graphed). In the graph is only the number of mosquitos at the warm side plotted (the 'control' temperature, room temperature, was set to 22.4°C). The amount of individuals at the cold side did not chance over time, therefore only the warm side (40°C, bold red) was graphed. With 40°C at 60 seconds, approximately 30 mosquitos navigated to the warm side. At 300 seconds oriented 45 mosquitos to the warm, IR-emitting source. The higher temperatures, e.g. 80°C, increased the mosquitos' ability to navigate to the warm plate. With 80°C navigated 15 mosquitos at the start to the warm side, at 150 seconds oriented over 50 mosquitos and at 300 seconds approximately 45 individuals. At 34°C, a constant amount of 10 mosquitos was observed over the 300 seconds time span. In comparison stands 36°C with 10 individuals at the start, increasing to 22 at 150 seconds and finally 30 mosquitos at 300 seconds. The color-code ranges from dark blue (22.4°C) over light blue, purple and red (40°C) to black (80°C). **B** shows the temperature response through all performed experiments at the 5 minute time point. The 'control' with 22.4°C is blue colored, 40°C experiment red and all the other temperatures have black borders. The result shows that 40°C is the temperature attracting the highest number. The experiment was repeated once (sample size 1).

### 3.7 Long-term Heat-sensing of Mosquitos

To test whether *Aedes aegypti* mosquitos have a time-dependent limit in IR sense, a 12h long time assay was performed.

The result (Fig. 33) shows at 1 hour that 80 mosquitos navigate to the warm side, whereas 10 individuals navigated to the cold side. The high number of mosquitos at the warm side stayed constant until the time point of 7 hours. At 8 hours the number of individuals decreased from 80 to 45 mosquitos. After the decrease, the amount of mosquitos at the warm TEC increased until 12 hours to more than 90 mosquitos. For the cold TEC, the number of mosquitos ranged between 15-25 individuals over the entire time course of 12h. The difference of mosquitos between the warm and cold side remained constantly high throughout the entire experiment. For instance, the difference between the two TECs at 1 hour was 70 mosquitos, decreased to 25 mosquitos at 8 hours and increased at the end up to 75 mosquitos. The constant difference of mosquitos over time orienting towards the warm source showed that there is no time-dependent limit of thermal sensing.



**Figure 33 Long-term mosquito navigation towards an IR-emitting warm plate at 2 cm distance**

The figure shows the long-time mosquito navigation experiment with mosquitos orienting to either a 40 °C warm source (red) or a 22.8 °C cold source (blue) over a time course of 12 hours. The warm and cold plates were continuously (12 hours) set at constant temperatures. At 1 hour, the result shows that 80 mosquitos navigated to the warm side. The numbers of mosquitos at the warm side stayed constant until the time point of 7 hours. From 7 hour onwards, a decrease from 80 to 45 mosquitos was observed. After the decrease the amount of mosquitos at the warm TEC increased until at 12 hours (end of the experiment) to over 90 mosquitos. The

cold side, as symbolizing room temperature and therefore the surroundings, shows throughout the 12 hour time span a constant number of mosquitos ranging around 15-25 individuals. The experiment was repeated once.

### 3.8 Heat-sensing and the Effect of the DEET on Mosquitos

To test whether the repellent DEET interferes with the IR sensing in *Aedes aegypti* mosquitos, a cumulative DEET concentration-response experiment with 0.5  $\mu\text{L}$  steps was performed.

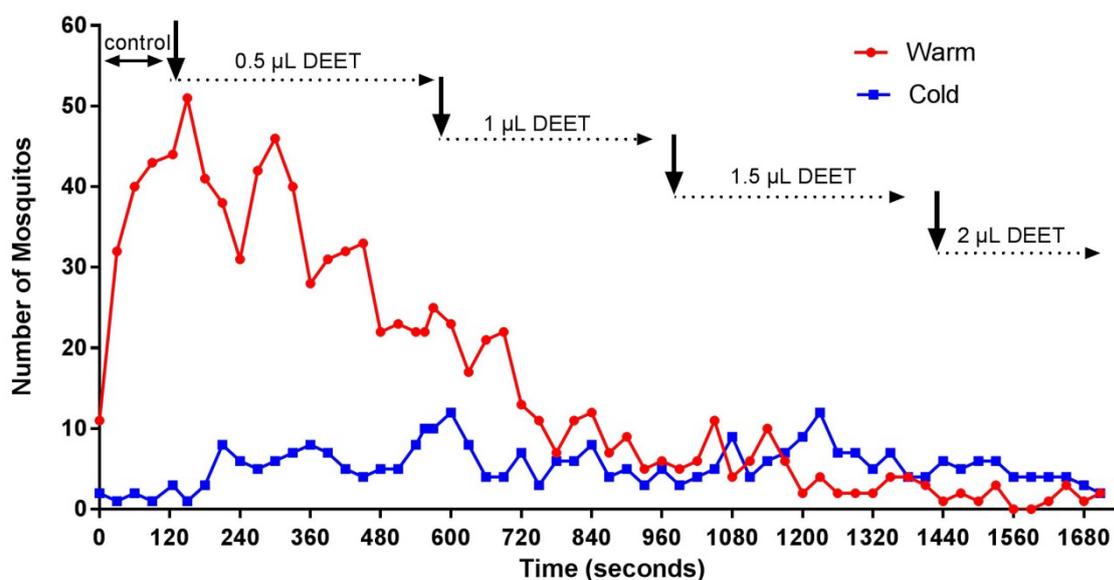


Figure 34 Action of accumulative DEET concentration on mosquitos IR sense

The figure shows the number of mosquitos that orient to either a 40 °C warm source (red) or a 22.8 °C cold source (blue) over a time course of 28 minutes (1680 seconds). From the start (part: control), the number of mosquitos orienting to the heat source (47°C) increased from 11 to 43 mosquitos. At 60 seconds, a difference of 38 individuals between warm and cold source was observed. After the successful navigation to the warm side, the first dose of 0.5 $\mu\text{L}$  DEET was evaporated at 120 seconds (first vertical black arrow). With 0.5 $\mu\text{L}$  DEET (0.000708 ppm) in the air, the number of mosquitos at the warm TEC decreased from 52 mosquitos at 120 seconds to 22 individuals at 480 seconds. The first DEET dose decreased the amount of mosquitos orienting to the warm side by half. After 1  $\mu\text{L}$  DEET evaporation (0.001416 ppm), the IR-sense was drastically disturbed. For instance, the numbers of mosquitos decreased from 22 at 580 seconds to 5 at 980 seconds. From 960 seconds onwards, no difference between the numbers of mosquitos navigating to either side of the TEC was observed, which means that DEET impaired the mosquito’s heat-seeking behavior. The DEET concentration was further increased until the final concentration of 2 $\mu\text{L}$  (0.002833 ppm). The cage used for this experiment contained 800 mosquitos and the experiment was repeated one time. The numbers above the arrow depicts the applied concentration, whereas the vertical arrows indicate the each 0.5 $\mu\text{L}$  vaporization time point.

First, the experiment (0-150 seconds) was done under normal conditions (warm vs. hot TEC) with the result that 40 mosquitos oriented at 60 seconds to the warm and 2 mosquitos to the cold side. The number at the warm side further increased to 45 at 120 seconds. After the results had proven that the tested mosquitos navigated to the warm TEC, the repellent was vaporized (arrow) with a soldering iron. At 120 s, the first 0.5 $\mu$ L DEET dose was evaporated into the 90 l chamber. The number of mosquitos aggregating in front of the warm side decreased from 52 at 120 seconds to 22 at 480 seconds. The first evaporation of 0.5  $\mu$ L DEET (0.000708 ppm) decreased the number of mosquitos at the warm side by half. In the next step, another 0.5 $\mu$ L DEET dose was evaporated, bringing the total to 1 $\mu$ L DEET (0.001416 ppm) causing the number of mosquitos at the warm side to further drop from 22 at 580 seconds to 5 at 980 seconds (end of 1 $\mu$ L trial). The attraction to the IR-emitting source decreased until no difference between the warm and the cold side was observed (960 seconds. This result showed that 1 $\mu$ L DEET (0.001416 ppm) eliminates the mosquitos' thermotactic behavior completely. A third 0.5 $\mu$ L DEET dose was added after 960 seconds, bringing the total concentration to 1.5 $\mu$ L DEET in the 90 l chamber (0.002125 ppm). The data shows again that there is no significant difference between the numbers of mosquitos between the two TECs. From 1200 seconds onwards, the warm side showed a lower number of mosquitos than the cold side. Again, 0.5 $\mu$ L DEET was evaporated, which increased the total DEET concentration to 2 $\mu$ L (0.002833 ppm) showing the same effect with no difference in numbers of mosquitos between the two TECs. Furthermore, preliminary experiments showed that mosquitos exposed to DEET until no orientation behavior also . Also did not respond to the hand.

The decrease of mosquitos navigating to the IR-emitting source and the lack of navigation to the human hand under DEET presence postulates a disruption of the thermotactic behavior. The effective dose was the evaporation of 1 $\mu$ L DEET (0.001416 ppm). It can be speculated that DEET further can be involved affecting the found IR-sense and therefore prevents mosquitos to orient to the warm source. However, this experiment provided a first insight into the action of DEET on disrupting the mosquitos' host-seeking behavior. The experiment was replicated once with 800 mosquitos.

### 3.9 Heat-sensing after Recovery from DEET Exposure in Mosquitos

To test whether *A. aegypti* mosquitos recover after DEET exposure in navigation to a warm source, an assay was developed to monitor the possible recurrence of the thermotaxis over a 16h experiment. Mosquitos were exposed to 2 $\mu$ L DEET (0.002833 ppm) at the beginning of the experiment until the thermotaxis was abolished. The arena was then ventilated and the

impaired mosquitoes were left in front of the two TECs over 16 hours. Before 2 $\mu$ L DEET was evaporated (Fig. 35), the mosquitoes were tested for the navigation behavior towards the warm and the cold TEC. At minute 2 (first data point, arrow) oriented 25 individuals to the warm side and 5 individuals to the cold side. Directly after the 2 minute time point, 2 $\mu$ L of DEET (arrow) was vaporized (0.002833 ppm), which was sufficient to block the ability of the mosquitoes to navigate to the warm source. After the compound treatment, the number of mosquitoes at the warm side dropped significantly to 1 mosquito. The mosquitoes' navigation towards the warm TEC was impaired from minute 2 until 14 hours. After 14 hours, the mosquitoes began to recover and started to navigate to the warm TEC again. At 16h, the mosquitoes were fully recovered with 40 individuals on the warm, IR-emitting source. This experiment showed that DEET can affect the function of the host-seeking behavior navigating towards a 40°C warm object over a time span of 14 hours. However, the full recovery of the mosquitoes' host-seeking behavior was at 16 hours.

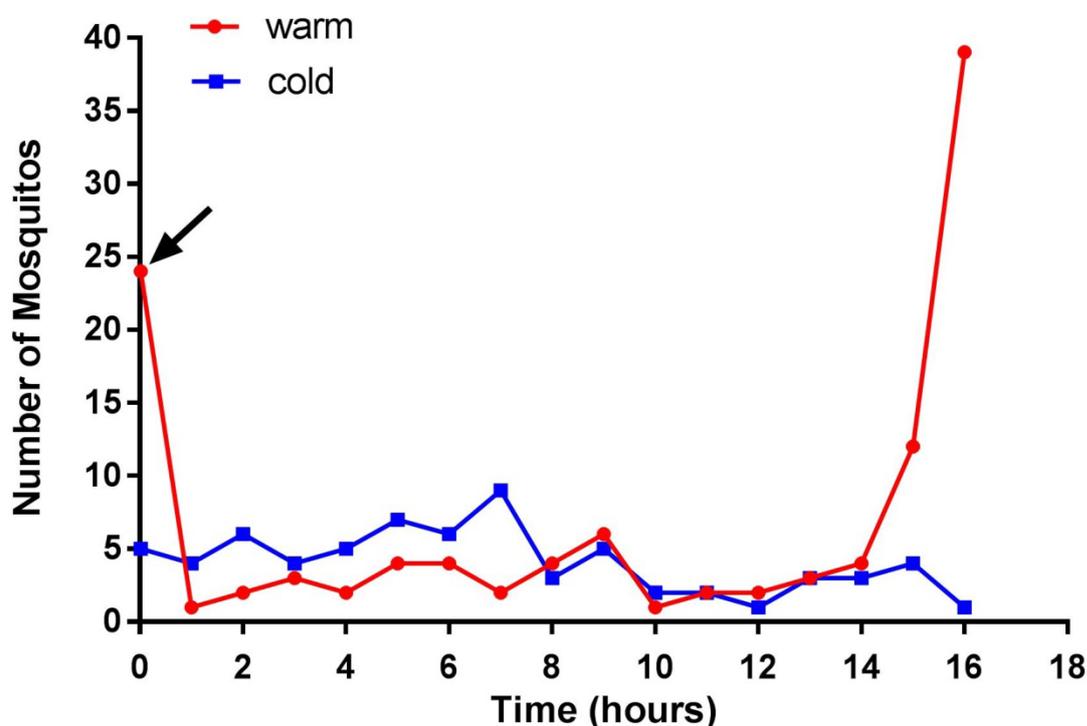


Figure 35 Recovery of DEET exposure on mosquito IR sense in *Aedes aegypti*

The figure shows the number of DEET-exposed mosquitoes that navigated over 14 hours to either the warm or the cold TEC. From the start, the hot side showed a difference of 25 mosquitoes at the warm (red) and 5 at the cold (blue) side. The first data point (0 at x-axis, indicated by arrow) represents minute 2 and shows that the mosquitoes were able to navigate to the warm TEC (warm vs. cold TECs). The IR navigating behavior decreased

after the vaporization of 2 $\mu$ L DEET (arrow; 2 $\mu$ L = 0.002833 ppm) after 2 minutes to almost 0 individuals. The navigation of the mosquitos was impaired from 2 minutes until 14 hours. From 14 hours onwards, an increase in mosquitos navigating to the warm source was observed. However, at 16h a total of 40 mosquitos navigated to the warm TEC (full recovery). The experiment was repeated once with 800 mosquitos.

### 3.10 Heat-sensing and the Effect of Citronellal on Mosquitos

The previous experiments with DEET and Cinnamaldehyde inhibited the navigation of mosquitos towards a warm IR-emitting source. Further Citronellal was tested to see whether the compound interferes with the mosquitos' host-seeking navigation behavior. The experimental setup was identical to the DEET experiment using the closed chamber to evaporate 5 $\mu$ L Citronellal (0.007526 ppm). Figure 36 shows the number of mosquitos that orient to either a 40 °C warm source (red) or a 22.8 °C cold source (blue). From the start of the evaporation, 30 mosquitos navigated at 60 seconds to the warm side, whereas 20 individuals to the cold side. The number of mosquitos at the warm side increased to 45 individuals until 160 seconds and 22 mosquitos at the cold side. After the mosquitos have oriented to the warm TEC, 5  $\mu$ L Citronellal (0.007526 ppm) was vaporized at 200 seconds (vertical arrow). The number of mosquitos at the warm side decreased to 18 mosquitos at 300 seconds. That shows a decrease of mosquitos of more than half within 100 seconds. At 480 seconds, the number of mosquitos did not differ between the warm and the cold TEC with 12 individuals each side. At 600 seconds, no difference in number of mosquitos between the warm and cold side was found. This result shows that the mosquito's heat-seeking behavior was impaired 400 seconds after the compound evaporation. From 600 seconds until 840 seconds (the end), no difference between the warm and cold side was observed. Overall shows this result that 5 $\mu$ L citronellal (0.007526 ppm) had an effect on the mosquito's heat-seeking behavior towards a warm, IR-emitting source. Thus, this experiment provided a first insight into the action of citronellal.

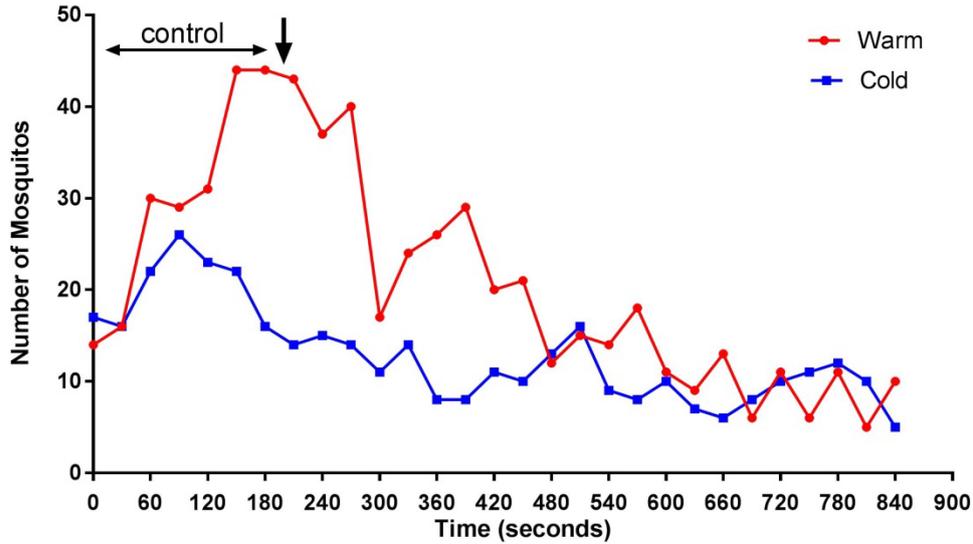


Figure 36 The effect of Citronellal on Mosquito's heat-seeking behavior

This graph shows the mosquitoes' orientation as a choice between a warm (40 °C, red) and a cold (22.8 °C, blue) TEC. Before the evaporation 30 mosquitos navigated at 60 seconds towards the warm TEC and later, at 160 seconds 45 individuals. The number of mosquitos at the cold side was also high with approximately 20 individuals at 60 seconds and 23 at 160 seconds. For the evaporation of 5µL citronellal (0.007526 ppm) was a soldering iron used (200 seconds, vertical arrow). The response to the warm TEC decreased by half to 18 mosquitos at 300 seconds. Between 300 to 420 seconds increased the number of mosquitos to 30 individuals. At 480 seconds, 12 individuals were counted at each TEC. Until the end of the experiment (at 840 seconds) no difference between the warm and the cold side was observed. This experiment was done once with 800 mosquitos per cage.

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### 3.11 Heat-sensing and the Effect of Cinnamaldehyde on Mosquitos

All the previous experiments have shown that the *A. aegypti* mosquitos are able to detect IR and orient towards an IR-emitting source. Further, it was tested if the mosquitos' host-seeking behavior is influenced by compounds affecting TRP channels (implicated in temperature and olfactory sensing). However, cinnamaldehyde is the most specific TRPA1 activator (Bandell, Story et al. 2004). A behavioral response assay with a warm and a cold TEC using compound vaporization in the closed chamber was performed. Figure 37 A shows the number of mosquitos that oriented under cinnamaldehyde exposure to either a 40 °C warm source (red) or a 22.8 °C cold source (blue) over the time span of 10 minutes. The experiment started out to test whether the mosquitos navigate to the warm side. After 60 seconds, the warm side shows between 40 to 48 mosquitos at the warm side and around 2 individuals at the cold side. At 120 seconds, 5µL cinnamaldehyde (0.001079 ppm) was evaporated using the soldering iron. The numbers at the warm side decreased over a time span of 30 seconds from 45 individuals at 120 seconds to 5 individuals at 150 seconds. The mosquitos started to recover after 150 seconds. After 330 seconds, half of the initial mosquitos' thermotaxic behavior was recovered with 25 mosquitos at the warm TEC which remained constant until 600 seconds. In comparison remained the number of individuals throughout the entire experiment at the cold TEC between 5 to 8 mosquitos.

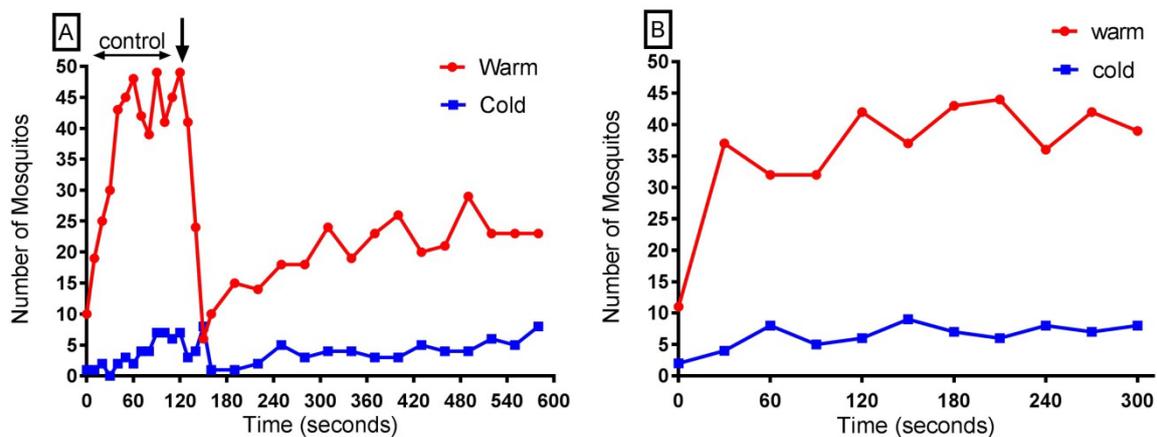


Figure 37 Effect of TRP channel activator Cinnamaldehyde on *Aedes aegypti* and the recovery

The graph A shows the number of mosquitos orienting to the warm side (40°C, red) and to the cold side (22.8°C, blue). After 60 seconds, between 40 to 48 mosquitos (see arrow control) oriented to the warm side, whereas the cold side ranged between 1 to 5 individuals. 5  $\mu\text{L}$  cinnamaldehyde (0.001079 ppm) was vaporized at 120 seconds (vertical arrow) and caused a sudden decrease of mosquitos that have been navigated to the warm side. The numbers of mosquitos decreased from 45 at 120 seconds to 5 individuals at 150 seconds. After 150 seconds, the mosquitos recovered quickly with 15 individuals at 180 seconds to 25 individuals at 300 seconds. Figure B shows the recovery experiment after 15 minutes (includes 5 minute arena ventilation) break with the mosquito cage at the lab bench. The number of mosquitos at both sides recovered quickly and at 30 seconds were 37 mosquitos at the warm side and approximately 5 individuals at the cold side. The number of mosquitos that navigated to the warm side increased further to 45 mosquitos at 120 seconds. Throughout the experiment remained the cold side constant with 5 to 8 mosquitos. The experiment was done once with 800 mosquitos per cage.

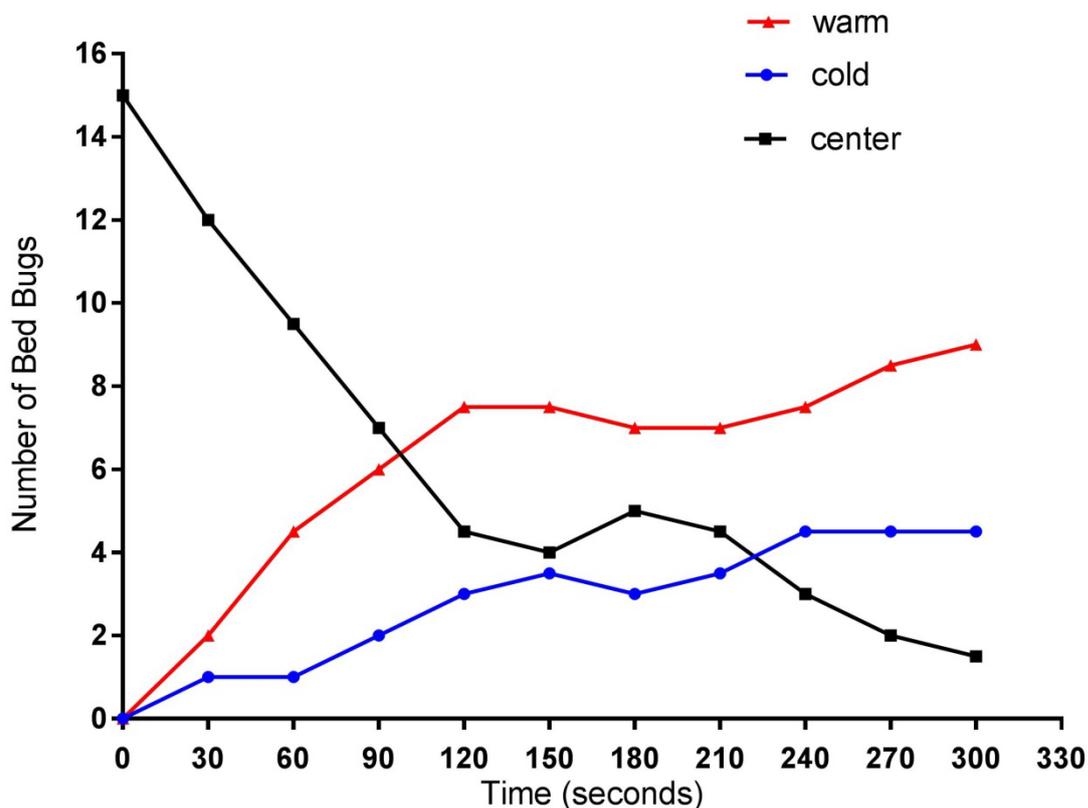
Before the recovery experiment started, the arena was well ventilated (5 minutes) with a 15 minute break (Figure 37 B). The number of mosquitos orienting to the warm side increased from the start with 10 mosquitos to 37 individuals at 30 seconds. At 120 seconds, approximately 45 mosquitos oriented towards the warm side, which is a similar to the number of mosquitos at 60 seconds in the compound evaporation experiment (Fig. 33 A). In the recovery experiment was the attractiveness of the cold side low with 5 to 8 individuals. The result showed that the navigation towards a warm IR-emitting source was impaired by 5  $\mu\text{L}$  cinnamaldehyde (0.001079 ppm). The recovery time was approximately 20 minutes for 5 $\mu\text{L}$  cinnamaldehyde.

## Results Bed Bugs

In order to test whether *C. lectularius* sense and make use of infrared radiation, an arena experiment (see Materials and Methods) was performed to test behavioral responses towards thermal sources. However, the bed bugs prefer the left side of the arena for unknown reasons. The reason for the left side preference of the bed bugs is not associated with the warmth of the source and therefore not sensed by the antennae.

### 3.11 Heat-sense in Bed Bugs with IR, blocked IR and amputated antennae

Figure 38 shows the number of bed bugs that orient to either the warm or cold side of the experiment over 300 seconds. Over time, more bed bugs moved to the warm side of the arena but due to the left-bias is no conclusion possible. At 150 seconds, 8 bed bugs moved to the warm side, whereas 4 bed bugs oriented to the cold side. By 300 seconds, approximately 9 individuals were observed at the warm source, compared to 5 individuals at the cold side. The data shows at 300 seconds a difference of 4 individuals between both sides. However, the mean distance (Fig. 41) of the three different experiments does not show a difference between the three experiments, which means that the bed bugs are not able to find the warm source. Overall, no difference between the three experiments (no difference between IR, no IR and antenna amputation) indicates that bed bugs do not detect IR.



**Figure 38 Average response of female bed bugs toward an IR-emitting warm plate**

The figure shows the experiment with the TECs kept at 22.8 °C (blue, cold) vs. 37 °C (red, warm). The graph is showing the number of bed bugs in center (black squares), warm (red triangles) and cold side (blue circles) (sample size = 30)

In the next experiment (Fig. 39), aluminum-foil was glued on the TECs surface to block IR-radiation. Thermocouple measurements and measurements with the infrared camera showed that surface and air temperature did not vary above ambient more than a few mm from the warm plate (data not shown), showing that the bed bugs were unlikely to be sensing the heat by convection or conduction. We also repeated the experiment with the warm plate covered with shiny aluminum foil, which would present a warm surface that reflects IR radiation of the surrounding surfaces. If the bed bugs' attraction to the warm surface would depend on IR sensing, then they should not be attracted to the foil-covered plate. In the experiment, both sides of the TEC were covered with aluminum foil. Figure 39 shows the number of bed bugs that oriented either to the 37 °C warm source (red) or the 22.8 °C (blue) cold source. No difference in number of bed bugs between the warm and cold side was observed. The mean distance (Fig. 41) of the three experiments does not show any difference between the three different experiments, therefore it can be said that the bed bugs are not able to find the warm source. This could mean that the bed bugs are not able to navigate to a warm source using

IR. However, the experiment was left-biased therefore no conclusion can be made.

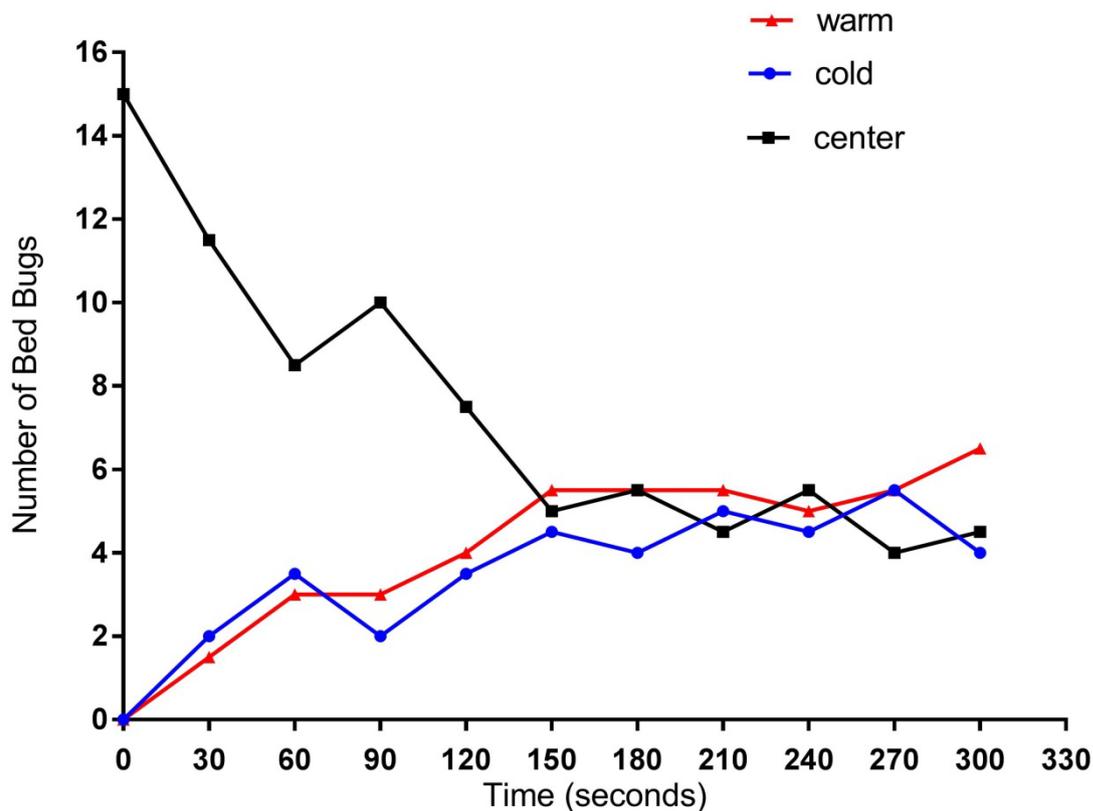


Figure 39 Average response of female bed bugs toward a non IR-emitting warm plate

The figure shows the experiment with the TECs kept at 22.8 °C (blue) vs. 37 °C (red) on either side of the arena. The graph is showing the numbers of bed bugs in center (black squares), warm (red triangles) and cold side (blue circles) (sample size = 30).

In the last experiment were both bed bugs' antennae amputated to test whether the bed bugs use their antennae to find the warm, IR-emitting source (Fig. 40). From the start until 150 seconds, no difference between both sides of the numbers of bed bugs was observed. After that, the number of individuals orienting to the warm side increased from 5 to 6 individuals. In contrast to that, the number of bugs on the cold side decreased from 4 to 3 individuals. From 150 seconds onwards, the number of bed bugs increased at the warm source, whereas fewer individuals were attracted to the cold source. Reasons for the increase are unknown.

However, the mean distance (Fig. 41) of the three different experiments does not show a difference between the three experiments, which means that the bed bugs are not able to find the warm source. Overall, there is no difference between the three experiments indicating that

bed bugs do not detect IR. The experimental setup was left-biased which did not allow a conclusion.

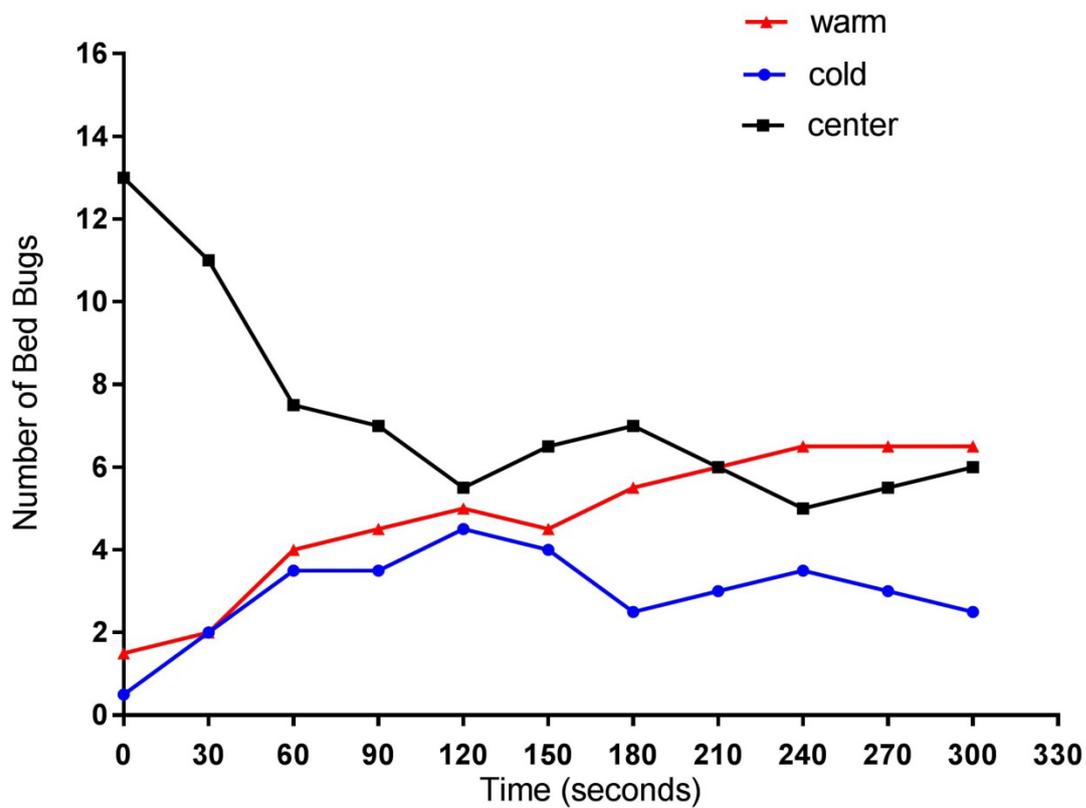


Figure 40 Average response of female bed bugs towards an IR-emitting warm source with cut antennae

The figure shows the experimental setup as usual with the TECs kept on 22.8 °C (blue) vs. 37 °C (red). The graph is showing the numbers of bugs in center (black squares), warm (red triangles) and cold side (blue circles) (sample size = 30).

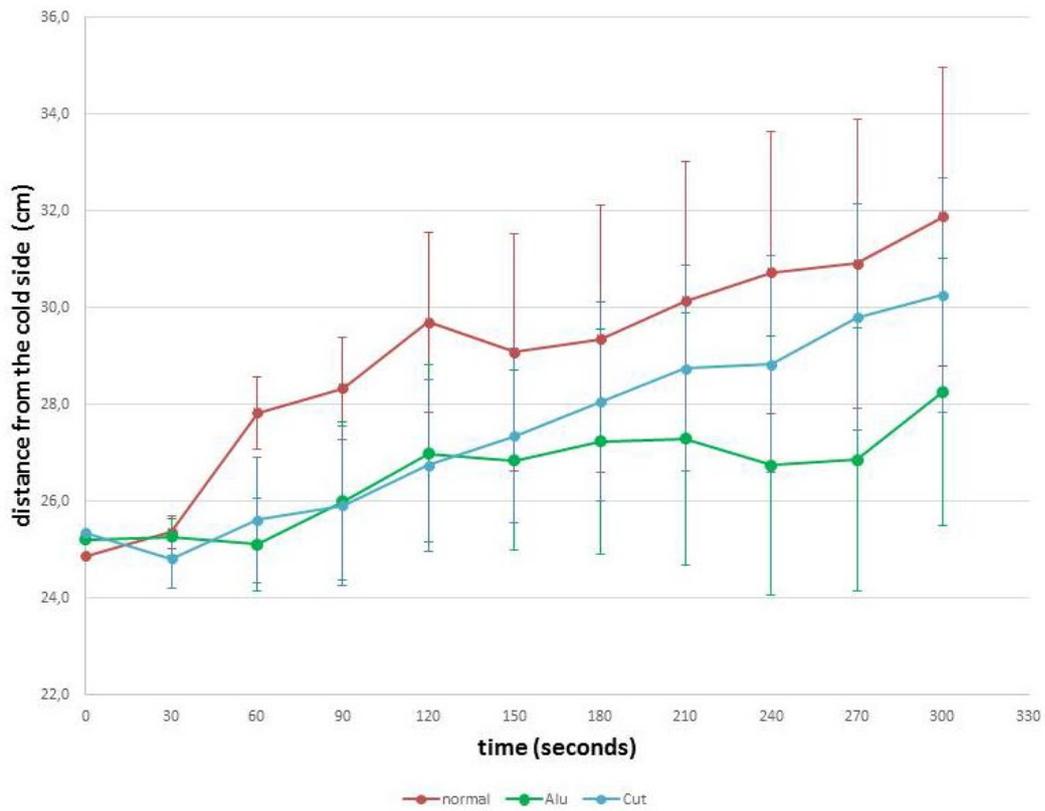


Figure 41 **Mean distance of bed bugs**

This graph depicts the mean distance of the three experiments (normal, alu and cut). The data shows that there is no difference in distance between the three experiments due to the large standard error of the mean.

## 4. Discussion

### 4.1 IR-sensing in Mosquitos

The literature review stated that mosquitos and bed bugs use temperature as a host cue to locate a source of nutrition. So far, it has been shown that mosquitos navigate to heat transferred by convection ((Peterson and Brown 1951), (Aboul-Nasr and Erakey 1967)). Furthermore, the molecular basis of heat sensitivity in *An. gambiae* was studied by Wang. Wang's results showed that small coeloconic sensilla at the distal tip of the antenna express genes of an anopheline TRP channel homolog. These sensory structures respond to temperature gradients, therefore also to convection. However, heat is transferred in three ways as convection (warm currents), conduction (heat transfer through contact) and radiation (transfer of energy by electromagnetic waves). We speculated that heat is transferred by radiation and therefore warms up the peg organ in the pit. Furthermore, TRP channels could be involved in heat-sensing to elicit the host-seeking behavior. In the experiment, the convection of the TEC heating-elements was eliminated by using Saran wrap. Saran wrap allowed the transmission (high transmissivity of 0.95-1) of IR radiation (checked with IR camera, data not shown), therefore we could say that the mosquitos detect IR. The results show that the mosquitos are attracted to objects at a distance of 2 and 5 cm at 40°C. This shows that mosquitos are able to navigate to an object of 40°C by using an IR-sense. The mosquitos also oriented the side of the warm source at a distance of 10 cm (Fig. 24) even if no statistics were raised due to the small sample size (n=2). It can be said that an added 3<sup>rd</sup> value (with an averaged value of the two samples) would have made the result significantly different, i.e. the number of mosquitos between the warm and cold side would have differed. The assumption that mosquitos can orient towards a source of a distance of 10 cm is supported by the ratio graph (Fig. 25). The experiment with a distance of 10 cm depicts a ratio of 1.5, which indicates that more mosquitos go to the warm side as, for example, in a non IR-emitting source (with a ratio = 1, aluminum-foil covered TEC). However, repeating the experiment with a proper sample size for statistical analysis would allow us to conclude that mosquitos detect the warm source from 10 cm. Blocking the IR radiation with an aluminum foil prevented the mosquitos from locating and navigating to the warm, IR-emitting source. Aluminum foil has a low emissivity of 0.04 (Saran wrap emissivity: 0.95-1) which blocks IR radiation but still heats up to 40°C. Therefore our result provided evidence that mosquitos are navigating towards an IR-emitting warm source and not to convection. Further, estimations of the maximum IR-sensing distance of mosquitos were done. Therefore, the ratio of mosquitos over time was plotted over the three distances (Fig. 27) and a linear regression line ( $Y = -$

$0.4642 * X + 5.533$ ) was added. However, if we assume that mosquitos also detect a warm source at 10 cm, then predictions of the detection limit of a bigger source (e.g. the human torso) could be estimated. If I compare my torso length (60-70cm, head to pelvis) with an IR source for mosquitos (vertical: 10 cm), then calculations would predict that mosquitos would be able to find the host from a distance of 60-70 cm (Fig. 42). Furthermore, if mosquitos recognize a warm source (real temperature: 40 °C, apparent temperature: 35.23 °C) at 10 cm distance then we could estimate that humans (32-35°C) could be found within a distance from 60-70cm. Simply put, the mosquitos perceive more IR emissions from a bigger body but these speculations disregard the fact that the IR intensity most probably decreases over the distance and that mosquitos possibly also use other cues like CO<sub>2</sub> and body odors (e.g. lactic acid) (Lazzari and Wicklein 1994) to find human hosts. The host-seeking also depends on other factors such as physiological states, for example, the reproductive status and diapause (Bowen 1991). This could especially be important as the physiological status needs to be taken into account for further experiments. However, we used starved mosquitos for our experiments.

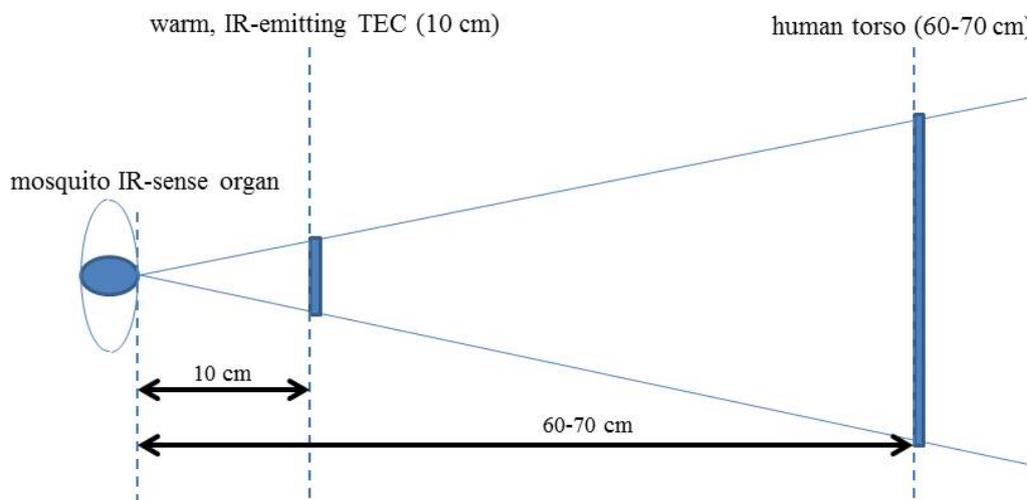


Figure 42 **The estimated mosquitos' field of view**

The figure shows the mosquito IR-sense organ at the left side (circle), the warm, IR-emitting source of 10 X 10cm and the human torso with 60-70 cm (averaged value from humans). This graph estimates the distance of mosquitos recognizing a human body based on these thesis experiments.

The extended-time experiment has shown that *A. aegypti* mosquitos can navigate to a warm source over a time span of 12 hours (Fig. 33). A constant number of over 60 mosquitos at the warm side gave further evidence that there is no time-dependent limit of thermal sensing. No examples were found in the literature about long-time experiments; therefore the answer to the question why mosquitos navigate to the warm source over a time span of 12 hours remains

unknown. However, a possible reason could be that mosquitos cannot deactivate the IR-sense, i.e. the sensory organ will be stimulated as long as the IR stimulus is present. Further evidence of the IR-seeking behavior provided another experiment where both antennae were cut off. This experiment supports that the IR sensory organs are located at the antennae, as mosquitos without antenna were not able to find the IR-emitting source. The IR sensory organs are suspected to be the coeloconic sensilla, also called the 'peg in a pit' (Fig. 1). The reason for this assumption is that we think the organ works as a pinhole camera. Therefore, the pit can function to locate the IR source as a directory window, whereas the peg gets stimulated by the IR radiation when facing the source. A further look into the IR detection ranges in snakes gave insights into possible functions of the pit organs as the IR detection depends on the threshold (the minimum IR irradiance of an object against the background) at a certain distance (Ebert 2007). The function of the coeloconic sensilla perceiving convection has been shown in the host-seeking behavior in ticks, as they use their forelegs with the 'peg in a pig' organ to forage and locate the host (Lees 1948). Overall, we speculate that coeloconic sensilla are IR sensing organs. This speculation is supported by the fact that Wang found the expression of the anopheline homolog of TRP channels in the distal tips of *Anopheles* mosquitos and associated the structures being sensitive to temperature gradients (Wang, Qiu et al. 2009). We think that IR radiation, rather than convection, heats up the peg in the pit and therefore elicits the mosquitos' heat-seeking behavior. Further experiments of that thesis showed that only the female mosquitos are able to navigate to a warm IR-emitting source (Fig. 29 A). Literature states that, a known sex difference in mosquitos is that females carry modified mouthparts to feed on blood, whereas the males rely on nectar as the only source of nutrition (Zettel and Kaufman 2012). Therefore, the different feeding behaviors implement a difference in host-seeking and could explain why exclusively females were attracted to a warm source by IR-sensing.

An outlook calculating the percentage of oriented mosquitos, according to the total number, can be an estimator for the physiological state mentioned above. For example, in the experiment with a distance of 2 cm, 30 out of 800 mosquitos oriented at 300 seconds towards the warm source. All used cages had an approximate 1:1 ratio of females to males, therefore it can be said that 30 of 400 mosquitos (navigation rate: 7.5%) navigated to the warm side. The calculations are based on the female vs. male experiments which confirmed that exclusively female mosquitos are attracted to the warm side. In the experiment with a distance of 5 cm, 15 out of 400 mosquitos (navigation rate: 3.75%), and in that with a distance of 10 cm, 12 out of 400 mosquitos (navigation rate: 3 %) oriented to the warm source. The navigation rate of the

different sexes, as only 100 individuals were tested at each side, showed that 10 of 100 (10%) female mosquitos were attracted to the warm side. Overall, reasons for the high variation rate between the experiments are unknown, but it can depend on the physiological state. It is important to know is that the navigation rate is only an estimated value as the total number of mosquitos was not precisely counted.

In summary it can be said that our experiments laid the foundation for the assumption that mosquitos are attracted to objects at 40°C, which are detected through IR-sensing. However, previous literature stated that mosquitos perceived convection or can sense temperature gradients (Peterson and Brown 1951) but we have shown that they use IR radiation to navigate towards a warm source. Furthermore, a characterization of the newly discovered IR sense could be done with behavioral experiments by attraction to the warm TEC plate, or directly apply electrophysiological techniques at the mosquitos' antennae while varying the IR-intensity.

However, we have shown that mosquitos have an IR-sense which nobody has discovered before. The discovery can have other impacts on other blood feeding insects as often the host-seeking is partially or even not known. The literature states that mosquitos use a variety of cues, as for example heat, CO<sub>2</sub>, odor to find the host. Taking a closer look at heat perceptions showed that many studies reported convection as being responsible for host-seeking. These findings are definitely correct but detection of warm air currents is only possible on a close-range. Therefore, perceiving IR-radiation would allow the mosquitos a long-range detection as shown in the theoretical model recognizing a human body from 60-70 cm (Fig. 42).

## **4.2 Repellents**

### 4.2.1 DEET

The immense effect of DEET decreased the number of mosquitos navigating to the warm IR-emitting source. The results show that 1µL DEET (0.001416 ppm) was enough to disable the IR-sensing of the mosquitos towards the source of 40°C (Fig. 34). Furthermore, the first experiments with the DEET-treated mosquitos (exposure level between 2 to 5µL DEET) showed that mosquitos were not able to orient to a warm source and neither to the human hand (my hand close to the mosquitos screen). The following DEET recovery experiment showed that if the mosquitos are being exposed to 2µL DEET (in 90.09l arena, 0.002833 ppm) they need a minimum of 14 hours to recover. According to an internet research, 5 to 10% DEET protects for 2 to 4 hours, whereas 100% DEET repels mosquitos for over 10 hours (VertellusSpecialties 2016). The Environmental Protection Agency (EPA) published a review

stating that DEET is stable but sensitive to light and therefore shows rapid photo-oxidation via hydroxyl radicals. However, the estimated half-life ranges between 10 and 15 hours (EPA 2014). These literature findings, together with our results, show that DEET is effective over 10 hours. The repellency effectiveness is comparable to our results as we observed a minimum of 14 hours, even if the experiment was performed under controlled laboratory condition. Other available studies mentioned the hypothesis that DEET interferes with the detection of lactic acid (Syed and Leal 2008) or that DEET masks the olfactory system by decreasing the electrophysiological responses to 1-octen-3-ol. Overall, our data shows that DEET (0.001416 ppm) disrupts the thermotactic behavior of the mosquitos. The fact that DEET-treated mosquitos were not able to navigate to a human hand provided further evidence that DEET impairs the mosquitos' host-seeking behavior of orienting to the warmth. The likelihood that DEET influences many other organs (for example olfactory organs) cannot be ruled out, but the compound could affect the found IR-sense in the way that mosquitos lost the orientation to the warm source. This finding might open up discussions about the mode of action of DEET. Furthermore, electrophysiological studies could be done, for example, to find out if DEET affects the IR sense. If the discovered IR-sense is influenced by DEET, then further repellent development could help to improve, for example, the duration of the DEET effect as well as present a new field of research to find a new, more effective repellents. Additionally, more efficient repellents would help to decrease the disease spread in mosquitos of, for example, yellow-fever, dengue-fever, Zika virus.

#### 4.2.2 Citronellal

Citronellal also repels mosquitos from orienting towards the warm side, which allows us to postulate that there might be an influence of citronellal on the IR-sensing organs. The number of mosquitos decreased approximately 8 minutes after an exposure of 5 $\mu$ L citronellal (purity: 95%). However, the concentration of citronellal (0.007526 ppm) needed to affect the mosquitos was 5 times higher than with DEET (0.001416 ppm). No recovery experiment has been done for citronellal. Recent research by Kwon has shown that citronellal avoidance in *drosophila* was decreased with ablated antennae and maxillary palps. Therefore, Kwon suggested that the TRPA1 channels at the *drosophila* antennae are required for aversion to citronellal. In contrast to that stands *An. gambiae* where citronellal directly activates the TRPA1 channels with high potency (Kwon, Kim et al. 2010). These research studies let us postulate that citronellal could also activated the *A. aegypti* TRPA1 channels with the result of inhibiting the IR-sensing behavior of mosquitos. This work is important for repellent development as citronellal is a

natural occurring repellent and therefore obtains a better public acceptance than, for example, DEET. Furthermore, electrophysiological studies would help to gain more knowledge about the citronellal's mode of action which could help to determine a more effective, natural-based mosquito repellent.

#### 4.2.3 Cinnamaldehyde

Cinnamaldehyde inhibits the mosquitos' thermotaxic behavior as well. Cinnamaldehyde shows its biggest effect shortly after evaporation (Fig. 37) lasting not longer than some seconds before the mosquitos already started to recover. The mosquitos slowly recovered to half of the initial response within 2 minutes. The recovery experiment showed that the mosquitos' IR-sensing ability was restored 22 minutes after the evaporation of the compound. The recovery time of cinnamaldehyde is 22 minutes, whereas DEET-exposed mosquitos need 720 minutes (12 hours, Fig. 35) to recover. These findings indicate that DEET (1 $\mu$ L, 0.001416 ppm) is more potent than the tested cinnamaldehyde (5 $\mu$ L, 0.001079 ppm), however both have an effect at the mosquitos' heat-seeking behavior. Literature states that cinnamaldehyde is known to activate TRPA1 channels (Bandell, Story et al. 2004), which is interesting as we suggest that TRPA1 channels are involved in IR-sensing. Overall, our experiments showed that cinnamaldehyde temporary impairs the mosquito thermotaxic behavior. This could further suggest that the compound also affects the discovered IR-sense. Additional effects of cinnamaldehyde on the olfactory system cannot be ruled out, especially due to the strong smell. More experiment as, for example, electrophysiology would be required to prove the effect of DEET on the IR-sensory organs. This finding could open up new ways for research. A potential possibility could be to prolong the effectiveness of cinnamaldehyde by modifying the chemical structure or use it in combination with other repellent compounds. Therefore, cinnamaldehyde is important in repellent development. However, our experiment showed for the first time that cinnamaldehyde is able to interfere with the mosquitos' host-seeking behavior.

### **4.3 Bed bugs**

Bed bugs need to feed on blood in order to produce eggs (Reinhardt and Siva-Jothy 2007). However, before feeding on blood, the host needs to be located. So far the literature states that bed bugs show thermotactile behavior towards, for example, a human hand (Rivnay 1932). Furthermore, bed bugs have similar structures to coeloconic sensilla, as found in mosquitos (Olson, Moon et al. 2014), that could indicate that these structures are involved in host-seeking

of IR. In order to verify this hypothesis, behavioral experiments were conducted by using two TEC elements at different temperatures (warm vs. cold). The bed bug experiment showed an unknown preference for the left side of the arena. Reasons for that are unknown and not associated with the warmth of the IR-source and not sensed by the antennae. An additional graph (Fig. 41) depicts that there is no difference in the mean distance between the three experiments (warm vs. cold, IR-reflective warm and cold TECs (aluminum foil) and cut antennae (cut)). The fact that the three results are all the same shows that bed bugs do not detect the warmth, therefore do not detect IR. However, even if the other three graphs show that more bed bugs were found at the warm side, no conclusion could be made as the experiment was left-biased. Reasons for the bias could be, for example, that bed bugs left odor traces. In order to accommodate for that influence, the chamber was cleaned with 70% ethanol after every trial but traces could have remained. Another reason could be that the bed bugs were able to perceive the light of the hallway even if the small door window was light-tight covered with a cardboard. Furthermore, the hallway light could have affected the bed bugs while entering the room before conducting the experiments. The camcorder could have influenced the bed bugs as well as the night vision mode was used with an IR-emitting LED to film the experiment. However, a technician of Bell and Howell confirmed that the wavelength of the LED emits at approximately 750  $\mu\text{m}$  (near-IR). However, the thermal imaging region (far-IR) has a higher band, therefore a possible influence could be ruled out. Another reason of the left-bias could be that two bed bugs were simultaneously tested. This condition could have influenced the outcome of the experiment in a way that both insects could have influenced each other in finding the source. In order to prevent a left-bias in future experiments, bed bugs could be continuously stored in light-tight containers. This method will prevent prior light or odor exposure.

## Summary

Mosquitos and bed bugs are ectoparasites that use blood of humans and other species as a source of nutrition. In order to find the human, the ectoparasites are able to perceive several host cues, such as warmth, odor and CO<sub>2</sub>. We focused on warmth as a host cue, and so far, literature has provided evidence that the two tested ectoparasites can sense heat on a short-range by convection. The research methods made it possible to discriminate the mosquitos' behavior to a warm IR-emitting source from a distance. In other experiments, blocking IR-radiation with aluminum foil as a blocker inhibited the mosquitos from sensing the warm source, therefore did not allow host-seeking. Furthermore, mosquito experiments were done between female and male navigation behavior with the result that only female mosquitos found the warm source. This discovery could be associated with the fact that female mosquitos exclusively feed on blood. The navigation behavior towards a warm source at a different temperature was investigated in order to find the optimal host-seeking temperature. The optimal attracting temperature was an object with 40°C. By absorbing some of the IR radiation, the mesh material of the cage decreased the apparent temperature of the object from 40°C to 35.23°C (IR camera measurements), which is similar to the human body temperature. Furthermore, the orientation over an extended period towards a warm TEC showed that mosquitos do not desensitize in orienting to the 40 °C source. Finally, the effect of the three repellents (DEET, cinnamaldehyde and citronellal) was tested on mosquitos. DEET (97 % purity) had a strong effect in preventing the mosquitos navigating to the warm source. The recovery time of DEET was more than 12 hours, which shows that DEET impairs the mosquitos' thermotaxic behavior for a long period of time. Cinnamaldehyde also inhibited the mosquitos from finding the warm source with a recovery time of 22 minutes, therefore we concluded that the compound also affects the mosquitos' thermotaxic behavior. The last repellent, citronellal, also inhibited the mosquitos from finding the warm side, which shows an effect on the mosquitos' thermotaxic behavior.

Overall, the results for the mosquitos show that they are able to navigate to an object at 40°C (IR-emitting source). The navigation behavior was impaired by the removal of the antennae and the block of IR with aluminum foil. The bed bug results did not show any attraction to a warm source; however the experiment was left-biased. We have been able to support evidence that mosquitos use an IR-sense to detect a warm source of 40°C. The repellent experiments also affected the mosquitos' ability to find a warm IR-emitting source and therefore showed that the compounds affect the mosquitos' thermotaxic behavior. An effect of the repellents on the IR-sense could not be proven but can be speculated.

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## Eidesstattliche Erklärung

Ich versichere, dass ich die vorliegende Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe, alle Ausführungen, die anderen Schriften wörtlich oder sinngemäß entnommen wurden, kenntlich gemacht sind und die Arbeit in gleicher oder ähnlicher Form noch keiner anderen Prüfungsbehörde vorgelegen hat. Ich stimme zu, dass die vorliegende Arbeit mit einer Anti-Plagiatssoftware überprüft werden darf.

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