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**Influence of temperature on host location
by vibrational sounding in two pupal parasitoids**

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³Kroder, S., Samietz, J., Schneider, D. & Dorn, S. (submitted)

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1. Summary

Ambient temperature is a fundamental abiotic factor influencing in particular small poikilothermic animals like insects. It holds the potential to affect physiological processes as well as key processes in interspecific interactions. Host location of insect parasitoids is of crucial importance in parasitoid-host relationships in which both sides have established different strategies in a competitive co-evolution. In relationships with host pupae concealed in plant substrate, short-range host location of parasitoids largely relies on mechanosensory cues, as available visual and chemical cues are limited. Some species of the hymenopteran families Ichneumonidae and Orussidae have evolved a certain host location mechanism – the so-called vibrational sounding – that allows the wasps to detect host pupae under substrates like plant material or something similar. This mechanism is used as a kind of echolocation in which self-produced vibrations are transmitted onto the substrate and perceived by subgenual organ in the tibiae. Temperature dependence is especially likely in mechanosensory host location mechanisms since they depend on physical activity of the insect as well as on physical features of the substrate media. The goal of the present study was to investigate the influence of temperature on vibrational sounding during close-range host location.

In the first part of the thesis, the temperature effect was tested on the success of mechanosensory host location. At different ambient temperatures, females of the temperate species *Pimpla turionellae* (L.) and tropical species *Xanthopimpla stemmator* (Thunberg) (Hym.: Ichneumonidae) were exposed to plant-stem models containing a mechanosensory cue – excluding visual and chemical cues. The temperature ranges in which the parasitoids use vibrational sounding reflect the thermal conditions of the species' habitats. The females of the temperate species responded positively to mechanosensory cue from 6 to 32°C, and of the tropical species from 22 to 34°C with optima at 18-20°C and 32°C, respectively. The precision of ovipositor insertions due to vibrational sounding was relatively high over the entire range in *X. stemmator* and only declined at extremely low and high temperatures in *P. turionellae*. At higher

temperatures, the tropical species detected the mechanosensory host mimic more precisely. The results suggest that the use of vibrational sounding is adapted to the temperatures of the climatic origins. The reliability of this mechanism is thermally affected as responsiveness and searching activity decrease below and above of the temperature optimum and precision diminishes at the extremes.

The temperature dependence of vibrational signals was measured by a laser-Doppler vibrometer in further experiments. Vibrations produced and transmitted onto a plant-stem model by the temperate species *P. turionellae* were analyzed in the time and frequency domain. The time parameters did not change between ambient temperatures from 8 to 26°C, while the frequency and intensity were inversely affected as both increased with decreasing temperature. The wasps seem to deal easily with producing signals with high quality despite of thermal changes. The inverse shift of frequency and intensity is supposed to compensate a negative influence of lowered temperature on the receiving receptors. This would represent a further kind of “temperature-coupling” system to keep the functioning of short-range host location. The constant durations of time parameters and the increase in frequency parameters of self-produced vibrations further suggest a possible balance of body temperature in a changing thermal environment. Therefore, body temperatures of *P. turionellae* were recorded using real time infrared thermography, and wasps were simultaneously observed in their behaviour. Differences between females with and without vibrational searching behaviour were examined for their significance. Discrepancies of body temperatures to ambient surface temperatures were also compared between different thermal environments. Although there was no evidence for thermoregulation in searching females, pausing, i.e. non-searching females showed a significant regulation of body temperatures. Further, the wasps more often interrupted their searching behaviour at suboptimal temperatures. Vibrational sounding seems to interfere with endothermic mechanisms because low-level thermoregulation is only present in non-searching females. It is supposed that the searching parasitoids regularly pause during vibrational sounding to regulate body temperatures.

In a last experimental part of this thesis, the multisensory use of thermally sensitive vibrational sounding and thermally insensitive vision under optimal and suboptimal conditions were evaluated. Females of *P. turionellae* were tested in choice experiments with plant-stem models containing a visual and a vibrational cue at 8, 18 and 28°C. The relative importance of both cues was comparable at the intermediate temperature of 18°C. At the extreme temperatures of 8 and 28°C, the importance of the vibrational cue decreased in line with its lower reliability while that of the visual cue was maintained. The females switch between the use of multiple and single cues depending on the reliability and temperature dependence of the respective sensory systems. Moreover, the overall precision on the plant-stem models containing both cues did not vary between the tested temperatures, in contrast to the precision on plant-stem models containing only vibrational cue on which precision declined at 8 and 28°C (cf. above). Thus, the use of multisensory orientation not only improves the reliability of host location, it can also maintain the accuracy of host location at a high level even when ambient conditions impair single cues.

2. Zusammenfassung

Die Temperatur ist ein elementarer, abiotischer Faktor, der besonders auf kleine, poikilotherme Tiere wie Insekten einen starken direkten Einfluss hat. Sowohl physiologische Vorgänge als auch wichtige interspezifischer Wechselbeziehungen können durch die Temperatur beeinträchtigt werden. In Parasitoid-Wirts-Beziehungen ist die Wirtsfindung parasitoider Insekten von entscheidender Bedeutung. Beide Seiten der Beziehung haben in Co-Evolution verschiedene diffizile Strategien entwickelt, um sich an die andere Seite anzupassen. Wenn es sich beim Wirtsorganismus beispielsweise um ein in pflanzlichem Substrat verstecktes Puppenstadium handelt, ist der Parasitoid für die Wirtsfindung nach dem Landen auf dem Substrat in hohem Maße auf mechanosensorische Reize angewiesen. Visuelle und chemische Anhaltspunkte sind für den Parasitoiden in diesem Fall nur begrenzt verfügbar. Einige Arten der Familien Ichneumonidae und Orussidae (Hymenoptera) haben dabei einen Wirtsfindungsmechanismus entwickelt, der den Wespen ermöglicht, in Pflanzenmaterial oder ähnlichem verborgene Puppen aufzuspüren. Dieser Mechanismus ist eine Art Echoortung, wobei selbst produzierte Vibrationen auf das Substrat übertragen und mittels des Subgenualorgans an den Tibiaen empfangen werden. Dieser Mechanismus wird entsprechend als Vibrations-Peilung („Vibrational Sounding“) bezeichnet. Temperaturabhängigkeit ist bei mechanosensorischen Wirtsfindungsmechanismen besonders wahrscheinlich, da sie zum einen von der physikalischen Aktivität der Insekten und zum anderen von den physikalischen Eigenschaften des übertragenden Substrats abhängen. Ziel der vorliegenden Studie war, den Einfluss der Temperatur auf die Vibrations-Peilung zu erforschen, die von Parasitoiden zur Wirtsfindung nach der Landung im Mikrohabitat des Wirtes eingesetzt wird.

Im ersten Teil der Arbeit wurde der Temperatureinfluss auf den Erfolg mechanosensorischer Wirtsfindung untersucht. Weibchen der mitteleuropäischen Art *Pimpla turionellae* (L.) und der tropischen Art *Xanthopimpla stemmator* (Thunberg) (Hym.: Ichneumonidae) wurden an Pflanzenstängel-Modellen getestet, die einen mechanosensorischen Reiz enthielten und gleichzeitig visuelle

und chemische Reize ausschlossen. Die Temperaturbereiche, in denen die Tiere die Vibrations-Peilung einsetzen, spiegeln die Temperaturbedingungen ihrer natürlichen Habitate wieder. Die Weibchen der Art aus gemäßigtem Klima reagierten auf den mechanosensorischen Reiz bei Temperaturen von 6 bis 32°C mit einem Optimum bei 18-20°C. Die tropische Art reagierte bei Temperaturen von 22 bis 34°C mit einem Optimum bei 32°C. Ovipositor-Einstiche aufgrund der Vibrations-Peilung erfolgten bei *X. stemmator* über das gesamte Temperaturspektrum mit relativ hoher Treffgenauigkeit. Bei *P. turionellae* ließ die Treffgenauigkeit nur bei extrem niedrigen und hohen Temperaturen nach. Im höheren Temperaturbereich war die tropische Art treffsicherer als die Art aus gemäßigten Zonen. Damit legen die Ergebnisse die Schlussfolgerung nahe, dass die Vibrations-Peilung an die Temperaturverhältnisse der jeweiligen Klimazone einer Art angepasst ist. Die Temperatur beeinträchtigt die Zuverlässigkeit des Mechanismus, indem die Suchaktivitäten unterhalb und oberhalb des Temperaturoptimums nachlassen und die Präzision in extremen Temperaturen degradiert.

In weiteren Experimenten wurde die Abhängigkeit der Vibrationssignale von der Umgebungstemperatur anhand eines Laser-Doppler-Vibrometers gemessen. Vibrationen, die von Parasitoiden der Art *P. turionellae* produziert und auf das Modell übertragen wurden, wurden in zeitlichen Eigenschaften und Frequenzmerkmalen analysiert. In einer Umgebungstemperatur von 8 bis 26°C gab es keine Veränderungen in den Zeitparametern, während die Hauptfrequenz und die zugehörige Intensität sich umgekehrt zur Temperatur veränderten, d.h. Frequenz und Intensität stiegen bei sinkender Temperatur. Offensichtlich sind die Wespen trotz Temperaturschwankungen problemlos fähig, Signale in hoher Qualität zu produzieren. Die inverse Veränderung der Frequenz und der Intensität kompensiert vermutlich einen negativen Einfluss niedriger Temperatur auf die Rezeptoren. Ein solches Phänomen würde ein weiteres System einer „temperaturgekoppelten“ Signalproduktion und -verarbeitung darstellen, wodurch die Funktionalität dieses speziellen Wirtsfindungsmechanismus über eine weite Temperaturspanne aufrechterhalten werden kann. Die gleich bleibende Dauer der Zeitparameter und der inverse Effekt der

Frequenzparameter in den selbst produzierten Vibrationen lassen außerdem auf eine mögliche Stabilisierung der Körpertemperatur trotz variierender Umgebungstemperatur schließen. Daher wurde mit Echtzeit-Infrarot-Thermografie die Körpertemperatur von *P. turionellae* aufgenommen, während gleichzeitig das Verhalten der Wespen beobachtet wurde. Unterschiede zwischen Weibchen mit und ohne vibratorischem Suchverhalten wurden auf ihre Signifikanz geprüft. In gleicher Weise wurden auch Abweichungen der Körpertemperaturen im Verhältnis zur Umgebung unter kontrollierten Bedingungen in der Klimakammer verglichen. Weibchen mit Suchverhalten nach Wirten zeigten dabei jedoch keine Anzeichen für Thermoregulation, während pausierende, d.h. nicht suchende Weibchen, eine signifikante Regulation ihrer Körpertemperatur aufwiesen. Überdies unterbrachen die Wespen ihr Suchverhalten öfter bei suboptimalen Temperaturen als bei optimalen. Scheinbar stören sich die Mechanismen der Vibrations-Peilung und der endothermen Regulation der Körpertemperatur und schliessen einander aus, so dass Thermoregulation nur bei nicht suchenden Weibchen nachzuweisen ist. Folglich wird angenommen, dass suchende Parasitoiden immer wieder pausieren, um während der Vibrations-Peilung, die Körpertemperatur zu regulieren.

Im letzten experimentellen Abschnitt der Arbeit wurde die multisensorische Nutzung der temperaturempfindlichen Vibrations-Peilung und der temperaturunempfindlichen visuellen Orientierung unter optimalen und suboptimalen Bedingungen untersucht. Hierzu wurden *P. turionellae* Weibchen in Wahlexperimenten an Modellen getestet, die einen vibratorischen und einen visuellen Reiz enthielten. Solche Experimente fanden bei einer mittleren Temperatur von 18°C und bei extrem niedriger und hoher Temperatur von 8 beziehungsweise 28°C statt. Bei 18°C war die relative Bedeutung beider Reize vergleichbar groß. Jedoch bei 8 und 28°C, wo die Zuverlässigkeit des vibratorischen Reizes nachlässt, sank auch die relative Bedeutung des vibratorischen Reizes, während die Bedeutung des visuellen Reizes unverändert hoch blieb. Die Weibchen wechseln von der Wahrnehmung mehrerer Reize zur Wahrnehmung eines Reizes je nach Zuverlässigkeit und Temperaturempfindlichkeit der betreffenden sensorischen Systeme. Die Gesamtpräzision am Modell mit vibratorischen und visuellen Reiz unterschied sich nicht zwischen den untersuchten Temperaturen, im Gegensatz zu der Präzision an Modellen mit nur einem vibratorischen Reiz, die bei 8 und 28°C abnahm (s. oben). Folglich steigert die multisensorische Orientierung nicht

nur die Zuverlässigkeit der Wirtssuche, sondern hält auch die Genauigkeit auf hohem Niveau, wenn einzelne Reize durch Umweltbedingungen beeinträchtigt werden.

3. General Introduction

Direct relationships between species are the central issue to understand ecosystem functioning both in natural as in agricultural systems. Host location is a key process of the interspecific interactions in parasitoid-host relationships causing natural selection pressure on both sides that results in a highly competitive co-evolution (Begon *et al.*, 1998; Vet, 2001). While parasitoids explore several sources of information for host location, the hosts have established strategies to escape from parasitization (Godfray, 1994). The more complex the strategies of hosts are, the more cues of information should be involved by the parasitoids. In nature, host- as well as plant-derived cues are accessible in the searching process of parasitoid females allowing detection and recognition of potential hosts by chemosensory, visual and mechanosensory orientation. Volatile chemicals from insect hosts' saliva or frass as well as odours emitted from plants that are damaged by herbivorous host feeding are important cues for searching parasitoids (e.g. Vinson, 1998; Rott *et al.*, 2005). However, vibrational and visual cues, like patterns and colours, discolorations and entrance holes on infested plants or the motion and activity of hosts are also crucial components of host location (e.g. Michaud & Mackauer, 1994; Vinson *et al.*, 1998; Meyhöfer & Casas, 1999; Fischer *et al.*, 2003a). The use of many cues in multisensory orientation can improve the success of host location rather than discovering only one cue (Wäckers & Lewis, 1994; Battaglia *et al.*, 2000; Stireman, 2002).

Parasitoids which are pupae specialists have to overcome the challenge of reduced chemical and visual cues as pupae are immobile, do not feed and do not eliminate excrement. The release of chemical and visual cues is further limited if larvae pupate away from feeding site and hide under plant material or something similar (Vet *et al.*, 1991). For the successful detection of hidden hosts, pupal parasitoids are required to utilize a wider range of physical cues. Certain hymenopteran species have developed a particular mechanosensory mechanism to scent out hosts hidden in hollow space inside of plant material (Broad & Quicke, 2000; Vilhelmsen *et al.*, 2001). It is a kind of echolocation using self-produced vibrations instead of sounds that are transmitted by the antennae onto

the substrate. The subgenual organ, acting as mechanoreceptor on each tibia, perceives the vibrations and hypothetically allows the wasp to recognize solidity differences by comparing signals at the different legs (Otten, 2000). In analogous way to acoustics, this mechanosensory mechanism is referred to as vibrational sounding (Wäckers *et al.*, 1998).

Vibrational sounding is actually only accessible after landing on a potential host microhabitat and just available in short-range detection. Also, the use of chemical and visual cues can vary with the spatial scale depending on the accessibility and reliability (Wellings, 1993; Völkl, 2000). For example, plant odours are more important in the detection of the host microhabitat but less reliable after landing on the host plant (Vinson, 1998; Vet, 2001). According to this, changes of reliability, accessibility or detectability possibly lead to an altered relative importance in multisensory orientation. The importance of each cue determines how they interact between each other during the host location process. Detailed knowledge about the ecophysiological constraints of host location mechanisms is necessary for a better understanding of the searching procedure and the stimuli used by the parasitoids.

Furthermore, the reliability of certain host location mechanisms surely depends on environmental conditions. Temperature as one of the most important abiotic factors, especially in small ectotherms like insects, influences almost all biological processes (Willmer *et al.*, 2000). Thermal dependence is well known in acoustical and vibrational communication of arthropods and is presumed to affect likewise mechanosensory host location. Temperature is even relevant on both the producing and the receiving side of a vibrational system. Higher ambient temperatures usually result in an increasing frequency of signals like it is demonstrated in intraspecific communication of spiders (Shimizu & Barth, 1996), cicadas (Sueur & Sanborn, 2003) and field crickets (Pires & Hoy, 1992). Regarding the receiving side, mechanosensory receptors are substantially influenced by temperature in their physiological status during mechanotransduction, action potential encoding, and action potential conduction (e.g. French, 1985; Coro & Perez, 1990; Franz & Ronacher, 2002). Increasing temperature leads to a higher sensitivity to physical stimuli and improves the

temporal resolution in receptors and local interneurons (Coro & Perez, 1990; Franz & Ronacher, 2002). As a further matter, substrate-borne signals are transmitted with medium-specific attenuation which commonly varies with ambient temperature (e.g. Gogala, 1985; Greenfield, 2002). Thus, temperature influence could be assumed on the functionality of vibrational sounding affecting the host location activity and precision, the reliability, and its role in multisensory orientation.

To keep the functionality of vital processes in adverse thermal conditions, many insects have evolved strategies to maintain a balance of body temperature by ecto- and endothermic means. For example, body and wing colourations are adapted to absorb solar radiation elevating the body temperature (known as “basking”) (Forsman *et al.*, 2002). Therefore, the insects can behaviourally regulate their body temperature through microhabitat selection and arrangement to solar radiation, either to warm up or to cool down (e.g. Heinrich, 1993; Samietz *et al.*, 2005). The raising and maintaining of body temperature above the environment by endothermic means is particularly known in several hymenopteran species (Esch, 1988; Heinrich, 1993; Willmer *et al.*, 2000). The temporary endothermy (known as “heterothermy”) is well studied in honeybees and bumblebees (Heinrich, 1993) but has been also observed in other insect orders like Homoptera (Sanborn, 2001) and Lepidoptera (Esch, 1988). In the case of a thermal dependent host location mechanism, means of thermoregulation are also supposable, because successful parasitization is one of the most important elements in the life cycle of parasitoids.

The two species of the study are the temperate *Pimpla turionellae* (L.) and the tropical *Xanthopimpla stemmator* (Thunberg). Both are parasitoids of lepidopteran pupae, employ vibrational sounding to locate hidden hosts and are adapted to different temperature conditions. They belong to the subfamily Pimplinae (Hymenoptera: Ichneumonidae), which is the only taxon of Pimpliformes where endoparasitism has evolved (Wahl & Gauld, 1998). The species *P. turionellae* is widely distributed in temperate woodlands of Palaearctic (Bogenschütz, 1978), lives solitary and spends its entire immature life inside the host while consuming the complete contents of it (Sandlan, 1982).

The adults were already recorded early in spring in eastern Switzerland (Mani *et al.*, 1986) where quite low temperatures down to approximately 5°C can be experienced. By contrast, *X. stemmator* has a broad distribution in Palaeotropic (Hailemichael *et al.*, 1994) but a similar feeding and parasitization behaviour as *P. turionellae*. The species was successfully used for biological control of some lepidopteran stalkborers in the Old World. Whereas the host spectrum of *P. turionellae* contains also uncovered pupae, *X. stemmator* is specialized in stem borers (Meyer, 1925; Hailemichael *et al.*, 1994). Both species can easily be reared at pupae of *Galleria mellonella* (Lepidoptera), and according to the distribution ranges, they are very useful for a comparison of thermal behaviour in host location.

The thesis elucidates the effect of environmental temperature on host location by vibrational sounding and on its role in multisensory orientation. Primary, the success of mechanosensory host location was tested at different ambient temperatures in *P. turionellae* and *X. stemmator*, and compared between the two differently adapted species in an overlapping part of their temperature ranges (chapter 4 and 5). The effect of temperature on vibrational production of vibrations in *P. turionellae* was investigated in detail using laser-Doppler vibrometry and discussed with respect to a potential temperature-coupled adjustment of the signal intensity and frequency (chapter 6). A possible thermoregulation of the body was examined by real-time infrared thermography and simultaneous observations of the behaviour during host location (chapter 7). Finally, the relative importance of vibrational sounding in multisensory orientation and how the interaction with visual orientation altered was studied under optimal and suboptimal thermal conditions (chapter 8). It is hypothesized that the wasps accurately locate hidden hosts by vibrational sounding in a broad range of temperature reflecting the conditions of their natural habitats while they use specific strategies to cope well with thermal changes.

4. Ambient temperature affects mechanosensory host location in a parasitic wasp

Abstract. Certain parasitic wasps (Ichneumonidae, Pimplinae) use self-produced vibrations transmitted on plant substrate to locate their immobile concealed hosts (i.e., lepidopteran pupae). This mechanosensory mechanism, called vibrational sounding, depends both on physical cues of the environment and physical activity of the parasitoid and is postulated to depend on ambient temperature. We analysed the influences of temperature on vibrational sounding by choice experiments using plant-stem models with hidden host mimics in the temperate species *Pimpla turionellae*. The results show a significant effect of temperature on host-location activity and on the success of this process. Outside an optimum range, the performance of the wasps decreased both at low and high temperatures. Below 10°C and beyond 24°C the wasps displayed (1) substantial reduction in responsiveness, i.e. proportion of females showing ovipositor insertions, (2) reduction of quantitative activity with ovipositor insertions in the individuals, and (3) reduced precision of mechanosensory host location. Nevertheless, female wasps were able to locate their host over a surprisingly broad range of ambient temperatures which indicates that the wasps are able to compensate for temperature effects on vibrational sounding.

4.1 Introduction

Temperature is one of the most important environmental parameters influencing all organisms. Although thermal conditions affect nearly all biological processes, rates and functions (Willmer *et al.*, 2000), there are relatively few studies dealing with the influence of temperature on sensory mechanisms during species interactions. Most of this work is restricted to the influence of temperature on the

interaction between plants and the herbivores (e.g. Alonso, 1999; Bale *et al.*, 2002; Hughes *et al.*, 2004; Kührt *et al.*, 2005). Few studies are dealing with parasitoid-host interactions but are restricted to temperature effects on emission of potential behaviourally effective plant volatiles (Gouinguéné & Turlings, 2002; Vallat *et al.*, 2005), on the attractiveness of volatiles per se (Reddy *et al.*, 2002) or on parasitisation rates (e.g. Benrey & Denno, 1997; Flinn, 1998). How temperature effects relate to the sensory process of host location itself is mostly unknown. Accordingly, the importance of certain host location mechanisms in parasitoids under different temperature conditions is difficult to understand yet.

Parasitoids of concealed pupae depend heavily on physical cues as indicators of their hosts' whereabouts. Certain species of the Pimplinae (Hymenoptera, Ichneumonidae) have developed a strategy very similar to echolocation to locate their pupal hosts within the plant substrate or hidden in leaf rolls. They use self-produced vibrations transmitted by the antennae on plant parts to sense their immobile hosts (lepidopteran pupae) by signal modification received with the subgenual organ (Wäckers *et al.*, 1998; Otten *et al.*, 2002). This mechanosensory mechanism, termed vibrational sounding, depends both on physical cues of the environment and physical activity of the parasitoid (Fischer *et al.*, 2001; Otten *et al.*, 2002; Fischer *et al.*, 2003a, 2004a), and should therefore be related to ambient temperature: Since production of the vibrations during mechanosensory orientation is presumed to be of myogenic origin (Henaut, 1990; Otten *et al.*, 2002; Otten *et al.* unpublished); and the efficiency of mechanoreceptors is usually thermally influenced (e.g. Coro *et al.*, 1994; Hoger & French, 1999; Franz & Ronacher, 2002), the host location mechanism could be highly susceptible to changes of the wasps' body temperatures and changing ambient temperatures of the presumably ectothermic insects. It is to expect that the parasitoid females have a distinct temperature range according to the thermal conditions of their natural habitat in which they respond positively with host location by vibrational sounding. Within such an optimum range they should show high activity of ovipositor insertions towards the host which should decline if the temperatures rise too high or fall too low.

This suggests that also the precision of host location by vibrational sounding may decline outside optimum temperatures.

In this study, the possible influences of temperature on mechanosensory host location was analysed in the temperate wasp *Pimpla turionellae* (L.) (Hymenoptera, Ichneumonidae, Pimplinae). This polyphagous parasitoid of lepidopteran pupae has been well investigated with respect to the use of vibrational sounding as a host location mechanism (Wäckers *et al.*, 1998; Fischer *et al.*, 2001; Otten *et al.*, 2002) and with respect to physical environmental factors of the environment influencing the performance of this mechanism (Fischer *et al.*, 2003a). The temperate distribution of the species suggests a relatively wide range of ambient conditions the species has to deal with and it facilitates testing for performance during vibrational sounding from below 10°C to beyond 30°C. We tested the hypothesis of thermal influence of mechanosensory host location by choice experiments in the laboratory using plant-stem models with hidden host mimics. Such models elicit a behavioural response of the wasps which is comparable to host location in the natural system (Fischer *et al.*, 2004a) while excluding chemical and visual stimuli and therefore focusing solely on mechanosensory cues.

4.2 Material and methods

Parasitoid rearing

A laboratory strain of *P. turionellae* was reared on pupae of the wax moth *Galleria mellonella* L. Adults were kept in Plexiglas containers (25 x 25 x 25 cm) at 15°C and 70% relative humidity (rh) in a climate chamber (Conviron, PGV72, Winnipeg, Canada) with a photoperiod of 16h/8h:light/darkness. Wasps were fed with honey and water and are allowed to mate. Starting at an age of five to seven days in *P. turionellae*, parasitoid females were provided with host pupae for oviposition and host-feeding three times a week. After having been exposed to the wasps for three to five hours, parasitized pupae were stored at 24°C, 60% rh and a photoperiod of 16/8:light/darkness until emergence of adults, typically after

three weeks. Female *P. turionellae* have a lifespan of one to two months. The strain of *P. turionellae* was obtained in 1994 from the Forest Research Institute of Baden-Württemberg, Germany.

Plant stem model

Females of *P. turionellae* innately attempt to insert their ovipositors into various hollow rounded substrates due to their hosts' shelter inside of cocoons or leaf rolls. Paper cylinders containing a solid section imitating a plant stem with host pupa are therefore ideal experimental models and were used in a number of studies on host location in these insects (e.g. Wäckers *et al.*, 1998; Fischer *et al.*, 2001; Otten *et al.*, 2001; Fischer *et al.*, 2003a, 2004a). In the present study, the plant stem model was made of a hollow white cylinder (125 mm length; 8 mm diameter) made of airmail paper (ELCO, Atlantic Clipper Air Mail, 45 g m⁻², Allschwil, Switzerland). The mechanosensory cue of the host mimic was provided by a cigarette filter (15 x 8 mm, Gizeh, Gummersbach, Germany) hidden inside the paper cylinder. In this way, only mechanosensory cues are offered to the wasps, excluding chemical and visual stimuli on the model. Directed orientation with respect to the host mimic can therefore be attributed exclusively to vibrational sounding. This setup enables testing the responsiveness of the female parasitoids (i.e., the proportion of females inserting their ovipositor into the plant-stem model with the host mimic), the quantitative insertion activity by scoring the number of ovipositor penetrations, and the precision of host location by recording the position of insertions on the model in relation to the host mimic.

Experimental design

The tested females were at least one week old since emergence. Covered pupae were offered to the parasitoids for the first time 24 to 48 hours before the experiments started. At least one hour before the experiments animals were acclimatised to the ambient temperature of the according treatment. During the trials, single female parasitoids were placed individually into Plexiglas containers (18.5 x 8.5 x 7.5 cm) and exposed to one host model for a period of 20 minutes. The experiments were performed individually in a climate chamber

(Conviron, PGV72, Winnipeg, Canada) under high frequency white fluorescent light (10 kLux, 20 Wm⁻²) at 60% rh and the respective temperature treatment. All parasitoids were used for one trial session only. The stem model was attached to the white background and renewed in each trial. At the end of a trial the individuals were weighed with a high precision balance (Mettler Toledo MT 5, Göttingen, Germany; accuracy ± 0.01 mg).

Twelve temperature treatments with positive response of the wasps were applied in which 558 females were tested. The treatments were set from 6 to 24°C in 2°C increments, and at 28 and 32°C. Outside this range at 4 and 34°C no wasp showed a positive response with oviposition activity. The desired sample size for the treatments was at least 20 responding females, i.e. females that inserted their ovipositor at least once into the model during the trial period. At 10 and 20°C the sample size was increased to 30 responding females. The responsiveness at 6°C (30 females tested) and 32°C (36 females tested) was too low to reach the desired sample size. These two temperature treatments were therefore excluded from factorial analysis of quantitative insertion activity and precision of host location (cf. Data analysis).

Data analysis

The performance during host location was quantified by scoring the location and precision of ovipositor insertions on the stem-model in relation to the hidden host mimic. For the analysis, each stem-model was subdivided in 34 sections (section width: 3.7 mm). Host mimics were located in the 20th to 23rd sections.

Across the temperature treatments, the responsiveness of the species was analysed as the proportion of females inserting their ovipositor at least once into the stem-model. Quantitative insertion activity was analysed as the average number of ovipositor penetrations per responding female. Finally, the precision of insertions were compared between the temperature treatments by two parameters. First, absolute mean deviation of insertions from the cue centre (bias) for each responding female was calculated as the absolute value of the average insertions' deviation from the cues centre. Second, the variance of the insertion positions (scattering) was calculated for each female.

Differences in the general responsiveness of the females in the different treatments, i.e. the number of individuals inserting their ovipositor versus the inactive ones, were analysed using χ^2 -test. Quantitative insertion activity and the precision parameters were examined by analyses of variance (ANOVA). To test for a possible influence of body weight on the precision, the distribution of ovipositor insertions on the model was additionally assessed by an analysis of covariance (ANCOVA) with factor temperature and covariate body weight of the individual wasp. *Post hoc* pairwise comparisons were performed using Fisher's protected least square difference tests (Fisher's PLSD) on the 5%-significance level.

4.3 Results

Responsiveness

Over all treatments 40.0% of the tested female *Pimpla turionellae* responded positively to the plant-stem models with host mimic by ovipositor insertion. The temperature treatments with positive response ranged from 6 to 32°C (Fig. 1). At 4 and 34°C no wasp showed any insertion of the ovipositor. Within the range of positive response, ambient temperature had a highly significant influence on responsiveness of the females (χ^2 -test, $\chi^2=79.9$, $d.f.=11$, $P<0.0001$). At 18°C, wasps had the highest responsiveness with 69.0% of the tested females showing ovipositor insertions. Towards the low and high ends of the temperature range the responsiveness decreased to the lowest values at 6°C (13.3% response) and 32°C (13.9% response), respectively (Fig. 1).

Quantitative insertion activity

In the responding females, absolute numbers of ovipositor insertions were significantly influenced by the temperature treatment (ANOVA, $F_{9,209}=4.58$, $P<0.0001$). The highest average value was reached at 20°C with 7.40 ± 0.84 insertions (mean \pm s.e.). Towards the lowest and highest temperature with positive response, the activity decreased to 1.60 ± 0.80 insertions at 6°C and 1.80

± 0.58 insertions at 32°C (Fig. 2). Pairwise comparisons revealed a homogeneous group of average ovipositor insertions between 8 and 18°C without significant differences among each other (Fisher's PLSD, 5% significance level). The high averages at 20 and 24°C both as the low average at 28°C contrast significantly to this latter homogeneous group between 8 and 18°C (Fisher's PLSD, 5% significance level) (Fig. 2).

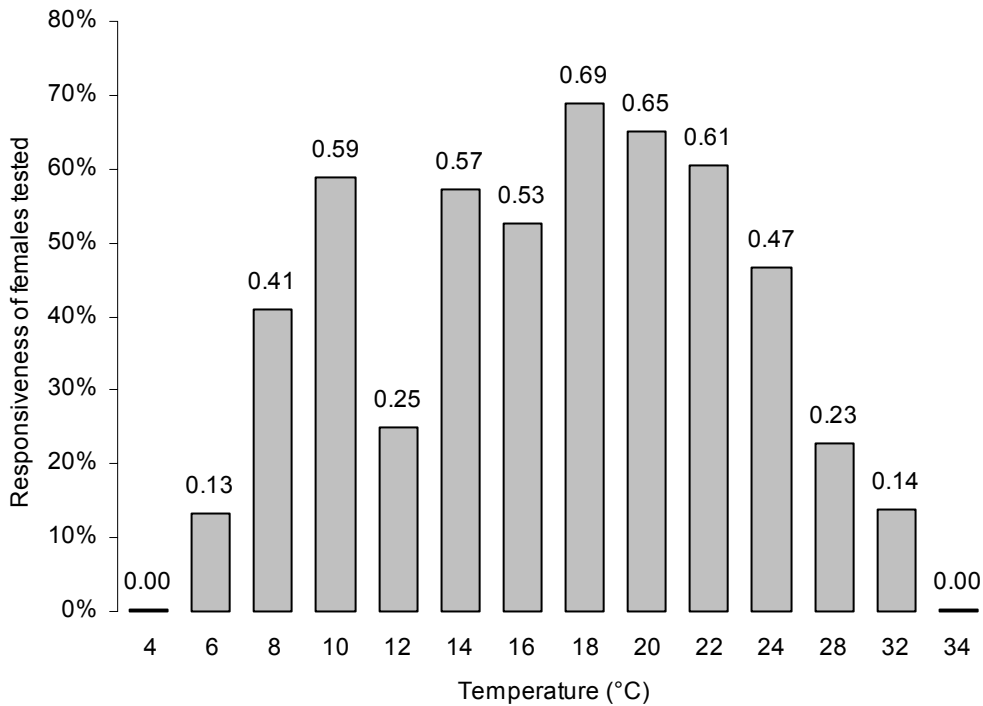


Figure 1. Responsiveness of *Pimpla turionellae* during mechanosensory host location on plant-stem models across temperature treatments from 4 to 34°C. Responsiveness is quantified as the proportion of females with at least one ovipositor insertion.

Precision of host location

The distribution of ovipositor insertions on the plant-stem models was strongly influenced by the temperature treatment but not by the body weight of the wasps (ANCOVA; factor temperature: $F_{9,209}=4.63$, $P<0.0001$; covariate body weight: $F_{1,209}=0.038$, $P=0.847$). Factor temperature did not reveal an interaction with the covariate body weight which indicates that the body weight of the female wasps does not modify the temperature influence (ANCOVA, interaction, $F_{9,209}=1.33$, $P=0.224$).

Quantified as the absolute mean deviation of individual ovipositor insertions from the cue centre, the bias of mechanosensory host location was significantly influenced by the temperature treatment (ANOVA, $F_{9,210}=4.54$, $P<0.0001$). The lowest average bias was found at 20°C with 1.19 ± 0.17 section widths (mean \pm s.e.). Towards the lowest and highest temperatures analysed, the bias of mechanosensory host location increased to 3.47 ± 0.77 section widths at 8°C and 4.23 ± 0.88 section widths at 28°C (Fig. 3 a). Pairwise comparisons revealed a homogeneous group of low average bias between 10 and 24°C without significant differences among each other (Fisher's PLSD, 5% significance level). The high average biases of host location at 8 and 28°C contrast significantly to this group (Fisher's PLSD, 5% significance level) and at the same time do not differ from each other (Fig. 3 a).

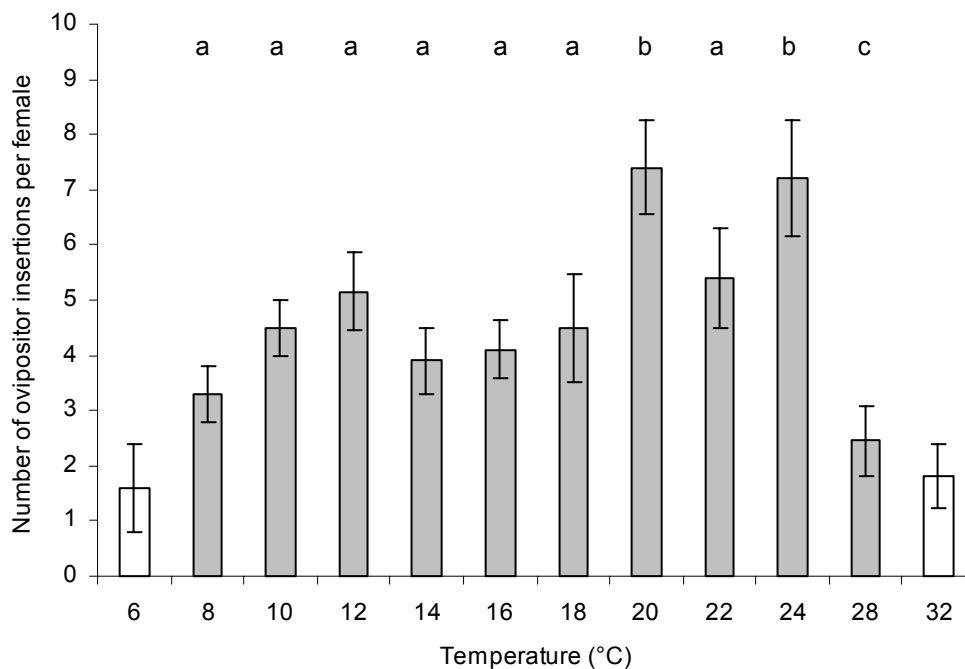


Figure 2. Quantitative insertion activity of *Pimpla turionellae* wasps during mechanosensory host location on plant-stem models across the temperature treatments (mean number of ovipositor insertions per responding female \pm s.e.). Different letters indicate significant differences in *post hoc* analysis (Fisher's PLSD, $P<0.05$) of the samples included in the ANOVA (grey boxes).

The scattering of mechanosensory host location (i.e., the variance of individual ovipositor insertions on the model) was also significantly influenced by the temperature treatment (ANOVA, $F_{9,166}=6.45$, $P<0.0001$). Lowest scattering occurred at 16°C with 9.58 ± 4.51 section widths (mean \pm s.e.). Similarly low values of scattering were found in all treatments between 12 and 20°C (Fig. 3 b). Towards the lowest and highest temperatures analysed, the scattering of mechanosensory host location increased to 17.42 ± 5.31 section widths at 8°C and 20.23 ± 7.24 section widths at 28°C (Fig. 3 b).

4.4 Discussion

The results of this study reveal a clear influence of ambient temperature (1) on the positive response during mechanosensory host location, i.e. the proportion of females inserting the ovipositor into the model with host mimic, (2) on the quantitative oviposition activity of the females, and (3) on the precision of vibrational sounding as host location mechanism in the parasitic wasps. Nevertheless the temperate species studied is able to maintain a high and constant performance of vibrational sounding over a surprisingly broad range of ambient temperatures.

Both responsiveness and quantitative oviposition activity of *P. turionellae* show an optimum around 18 to 20°C with decrease to lower and higher temperatures. These optima correspond roughly to the average temperature during the growing season in the temperate habitats of *P. turionellae* (Bogenschütz, 1978). At very low and very high temperatures of the range studied, responsiveness and the number of ovipositor insertions decline drastically. Below 6°C and beyond 32°C no response to the host mimic can be ascertained. Temperature limits for the sensory system are reflected by the drastically decreasing precision of ovipositor insertions both at the low and high limits of the temperature range with positive response to the host mimic. Both, bias of the host location mechanism and scattering of individual ovipositor insertions increase significantly below 10°C and at and above 24°C, respectively.

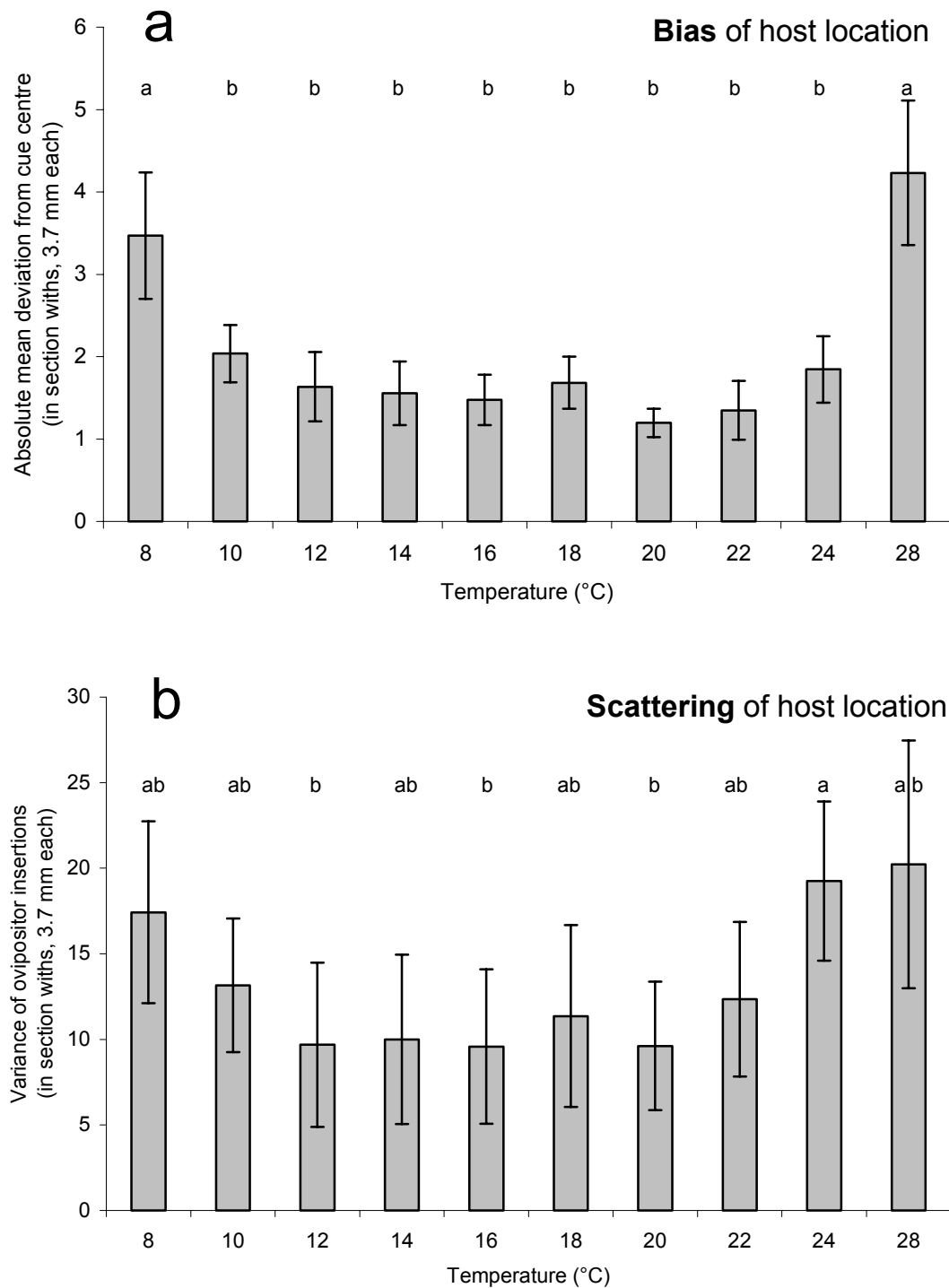


Figure 3. Precision of mechanosensory host location of *Pimpla turionellae* wasps on plant-stem models across the temperature treatments: Bias (a) quantified as the absolute mean deviation of individual insertions from the centre of the host mimic (expected value at 21.5 section widths); Scattering (b) as the individual variance of the insertions' positions. Different letters indicate significant differences in *post hoc* analysis (Fisher's PLSD, $P < 0.05$).

These results match our expectations that the quality of the sensory system declines outside an optimum temperature range to which the species is adapted. However, within a remarkably broad range of temperatures there is hardly any influence on the precision of host location. The bias of the mechanism is constantly low from 10 to 24°C. Prerequisites for such high precision of the sensory system are the ability to produce, receive, and compute signals for reasonably balanced orientation by vibrational sounding.

Hitherto little is known about the temperature influence on production and computation of vibratory signals in other arthropods. In spiders, higher ambient temperatures result in decreasing values of temporal parameters of substrate borne vibrations for intraspecific communication. However, there are also temperature-invariant parameters such as the duty cycle (Shimizu & Barth, 1996). Thoracic vibrations in *Melipona* bees correspond with the energetic status of the individual (Hrncir *et al.*, 2004a, 2004b). Therefore they may well also depend on ambient temperature but, to our knowledge, the latter has not as yet been investigated. Physically similar acoustical signals controlled by the neuromuscular system in arthropods are strongly influenced by ambient temperature especially in ectotherms (Gerhardt & Huber, 2002; Ryan & Kime, 2002). Higher body temperatures elevate the muscle contraction rates and allow faster oscillation of the muscles that generate the sound and hence produce higher frequencies (Greenfield, 2002). Myogenic sound production of crickets (Doherty, 1985; Martin *et al.*, 2000; Hedrick *et al.*, 2002) and cicadas (Sanborn & Mate, 2000; Fonseca & Revez, 2002), as well as the production of substrate borne vibrations in spiders (Shimizu & Barth, 1996) are subject to temperature dependence. The temperature ranges for sound production in insect species can be consequently quite small. Sub-tropical *Diceroprocta olympusa* cicadas sing at body temperatures within a range of just about 5 degrees above 35°C (Sanborn & Mate, 2000). In the temperate cricket *Gryllus integer* about 95% of males sing at habitat temperatures between 21 and 28°C (Hedrick *et al.*, 2002). On base of their temperature-dependence, the evolution of narrow ranges of males' song parameters through sexual selection can be attributed to stabilizing preference functions in females (Ritchie *et al.*, 2001). Additionally to the signal production,

in vibrational sounding, also the mechanoreceptor on the receiving side of the sensory system, the subgenual organ (Otten *et al.*, 2002), is most likely influenced by temperature. For arthropod mechanoreceptors, several studies report a thermal effect on mechanotransduction, action potential encoding, and action potential conduction (e.g. French, 1985; Hoyer & French, 1999; Franz & Ronacher, 2002).

Even though we can confidently state thermal effects both at the signal production side as on the reception side, the female wasps seem to deal efficiently with a wide range of changing temperatures during vibrational sounding. One possible explanation to achieve such performance with respect to temperature-variant vibration parameters could be temperature coupling between signal production and reception as known for acoustic communication during cricket- and grasshopper mate finding (Bauer & von Helversen, 1987; Pires & Hoy, 1992). A second explanation for the discrepancy between the expected temperature effect on vibration and the observed performance of the wasps might be the regulation of the body temperature permitting a correct functioning of vibrational sounding. In bees, for example, thoracic temperature is increased and well regulated by endothermic heat production with flight muscles (Esch & Goller, 1991; Heinrich, 1993; Hosler *et al.*, 2000; Stabentheiner *et al.*, 2003b). Whether or not the wasps studied here are able to alter their thoracic temperature by endothermy remains to be tested. Yet the results of this study improve our understanding of how temperature acts as a key environmental factor influencing the sensory physiology during species interactions and also contribute to the yet sparse information about the mechanism of vibrational sounding. In future studies, first, the vibratory signals should be investigated with respect to time- and frequency patterns under different temperature conditions, and second, the body temperatures of the wasps should be monitored during mechanosensory host location both at the limits as in the thermal optimum for vibrational sounding.

5. Effect of ambient temperature on mechanosensory host location in two parasitic wasps of different climatic origin

Abstract. Several parasitic wasps of the Pimplinae (Ichneumonidae) use self-produced vibrations transmitted through plant substrate to locate their concealed immobile hosts (lepidopteran pupae) by reflected signals. This mechanosensory mechanism of host location, called vibrational sounding, depends on physical characteristics of the plant substrate and of the wasp's body and is postulated to depend on ambient temperature. Adaptations of two parasitoid species to thermal conditions of their habitats and the influence of temperature on the trophic interaction during host location were investigated in the tropical *Xanthopimpla stemmator* (Thunberg) and compared with the temperate *Pimpla turionellae* (L.). Plant-stem models with hidden host mimics were offered to individual wasps under defined temperature treatments and scored for number and location of ovipositor insertions. Significant effects of temperature are found on host-location activity and its success. The tropical species possesses an optimum temperature range for vibrational sounding between 26 and 32°C, while the performance decreases both at low and high temperatures. The temperate species reveals substantial differences with respect to performance at the same thermal conditions. With increasing temperature, *P. turionellae* shows a reduced response to the host mimic, reduced numbers of ovipositor insertions, and decreased precision of mechanosensory host location. In the tropical *X. stemmator*, the female wasps are able to locate their host with high precision over a broad range of ambient temperatures, which suggests endothermic thermoregulation during vibrational sounding. Environmental physiology may therefore play a key role in adaptation of the host location mechanism to climatic conditions of the species' origin.

5.1 Introduction

Temperature is one of the most important environmental parameters for all organisms. It affects nearly all biological processes, rates and functions and its influence on metabolism and cellular biochemical processes is well understood (Willmer *et al.*, 2000). Temperature tolerance can vary considerably and has significant behavioural and ecological implications especially in poikilothermic animals like insects (e.g. Hausmann *et al.*, 2005; Samietz *et al.*, 2005).

Nevertheless, knowledge is limited about the influence of temperature on species interactions and, accordingly, on adaptations of prey- or host finding mechanisms in insects. Most of the respective current work is focused on the influence of environmental temperature on the interaction between plants and herbivores (e.g. Bale *et al.*, 2002; Richardson *et al.*, 2002; Kührt *et al.*, 2005). Particularly rare are studies considering the influences of varying temperature conditions on multitrophic systems or between herbivores and the highly important parasitoid guild. Some studies revealed significant effects of temperature on parasitisation rates (Benrey & Denno, 1997) or on the amount and quality of volatiles which are potentially effective on parasitoids' behaviour (Gouinguéné & Turlings, 2002; Vallat *et al.*, 2005). However, the consequences for host location by parasitic wasps and how the senses used during host location are affected are mostly unknown.

Successful parasitism requires an effective host location mechanism by the parasitoid. Depending on the senses involved, certain mechanisms of host location are more likely to be influenced by temperature than others. Influence of temperature is especially relevant in mechanosensory mechanisms of host location – like the vibrational sounding found in several species of hymenopteran parasitoids – because such mechanisms depend on physical properties of the environment and on the physical activity of the insects (Fischer *et al.*, 2001; Otten *et al.*, 2002; Fischer *et al.*, 2003a, 2004a). During vibrational sounding, female wasps transmit self-produced vibrations on solid plant material and locate their immobile pupal hosts hidden within the plant substrate by the sensed signal modifications (Wäckers *et al.*, 1998; Broad & Quicke, 2000). A direct

myogenic origin of the wasps' vibrations has been suggested from observations in previous studies (Henaut, 1990; Otten *et al.*, 2002; Otten *et al.* unpublished). Due to the fact that the power output of insect muscles is affected by temperature (e.g. Hosler *et al.*, 2000; Josephson *et al.*, 2000; Sueur & Sanborn, 2003), thermal dependence of vibrational signals during mechanosensory host location can be expected. Additionally, the vibrational signals are sensed with subgenual organs as mechanoreceptors in the parasitoid's tibiae (Otten *et al.*, 2002) and, because the efficiency of mechanoreceptors is also influenced by temperature (e.g. Coro *et al.*, 1994; Hoger & French, 1999; Franz & Ronacher, 2002), a temperature effect on the receiving side of the sensory system can be postulated.

Two species of pupal parasitoids (Hymenoptera: Ichneumonidae) with different climatic origins have been chosen here for testing the thermal dependence of vibrational sounding. *Xanthopimpla stemmator* (Thunberg) originated from the paleotropical regions of Asia and successfully colonized African as well as tropical American regions (Moore & Kfir, 1996). The species uses vibrational sounding in multisensory orientation and parasitizes lepidopteran stemborers (Fischer *et al.*, 2004a). *Pimpla turionellae* (L.) is widely distributed in temperate woodlands of the Palearctic (Bogenschütz, 1978) and has been well studied with respect to mechanosensory host location (Fischer *et al.*, 2001; Otten *et al.*, 2001; Fischer *et al.*, 2003a). According to the distribution, it is expected that the two species have distinct temperature ranges of effective vibrational sounding. In the present paper the temperature range in which *X. stemmator* uses vibrational sounding successfully was studied and a comparison made of two differently adapted species reflecting thermal conditions of the habitats to which they are adapted. The success of vibrational sounding was examined by offering plant-stem models with hidden host mimics to individual wasps excluding visual and chemical cues in laboratory treatments under controlled ambient temperatures.

5.2 Materials and methods

Parasitoid rearing

Laboratory strains of *X. stemmator* and *P. turionellae* were reared on pupae of *Galleria mellonella* (Lepidoptera). Adults were confined after emergence in Plexiglas® containers (25 x 25 x 25 cm) fed with honey and water and were allowed to mate. The wasps were kept at 23°C, 70% relative humidity (rh) in photophase and 15°C, 80% rh in scotophase with a photocycle of LD 16:8 h. The host pupae were exposed three times a week for ovipositing and host feeding. Parasitized pupae were stored at 24°C, 60% rh and a photocycle of LD 16:8 h until emergence of adults. The strain of *X. stemmator* originated from the University of Illinois at Urbana-Champaign, USA in 2000 and kept as explained above since then. The strain of *P. turionellae* was obtained in 1994 from the Forest Research Institute of Baden-Württemberg, Germany and kept at 15°C and 70 % rh with a photocycle of LD 16:8 h before the generation used in the present experiments.

Plant-stem model

The females of the two parasitoid species attempt to insert their ovipositor into various hollow rounded substrates. A paper cylinder (length: 125 mm, diameter: 8 mm) made of airmail paper (ELCO Atlantic Clipper Air Mail, 45 g m⁻², Allschwil, Switzerland) containing a cigarette filter (15 x 8 mm, Gizeh, Gummersbach, Germany) as solid section imitated the hidden host pupa. This experimental approach allowed solely mechanosensory cues to be offered, excluding chemical and visual cues. Hence, directed orientation can be attributed to vibrational sounding. Such plant-stem models have been used successfully in a number of studies on host location in these insects (e.g. Otten *et al.*, 2001; Fischer *et al.*, 2003a).

Experimental design

The females were tested after maturation which is reached typically after 25 days in *X. stemmator* females, and after seven days in *P. turionellae* females. The

wasps were allowed to mate prior to the experiments and had host contact for the first time 24 to 48 h before the experiments started.

The experiments were performed individually in Plexiglas® containers (18.5 x 8.5 x 7.5 cm) with one plant stem-model glued on a white background. Due to the distinct host niches the stem-models had to be positioned vertically in the trials with *X. stemmator* and horizontally in the trials with *P. turionellae*. At least one hour before the experiments the animals were acclimated to the test conditions, i.e. the respective temperature and 60% rh. Each trial lasted 20 min and at most five females were tested simultaneously in separate containers. All female parasitoids and stem-models were used only once. Accordingly, each model is associated with one female for the subsequent analysis.

Host location by vibrational sounding in *X. stemmator* females was investigated at seven temperature treatments from 22–34°C in 2°C increments. Outside this range, i.e. at 20 and 36°C, no wasp of the tropical species showed a positive response with oviposition activity. Exposure to temperatures of 38°C and higher during acclimation and the experiment (totalling to 1.5 h) led to a 100% mortality ($n=5$ females, S. Kroder, personal observation). The desired sample size of responding females was 20 for each of the seven temperature treatments. Furthermore as a basis for direct comparison of the tropical and the temperate species in the overlapping part of the temperature ranges, females of *P. turionellae* were tested for their performance in mechanosensory host location at 22, 26 and 30°C. Both species were reared under the same temperature conditions. The desired sample size of responding females was 30 for each species in each of the three temperatures allowing direct comparison.

A total number of 807 *X. stemmator* females were tested over the seven temperature treatments from 22 to 34°C. A total of 241 individuals of *P. turionellae* were tested for the direct comparison with the tropical species in the temperature treatments at 22, 26 and 30°C.

Data analysis

The success of mechanosensory host location by the parasitoids was quantified by scoring the number, location, and precision of ovipositor insertions on the plant-

stem model in relation to the hidden host mimic. The stem-models were subdivided into 34 sections (section width: 3.7 mm). The cigarette filter as host mimic was located in section 20 to 23. This allowed scoring of the position of insertions relative to the host mimic. For each treatment and species, the responsiveness as the proportion of ovipositing females, the quantitative insertion activity as the average number of ovipositor penetrations per responding female, and the precision of ovipositor insertions with respect to the host mimic using vibrational sounding as location mechanism were analysed. Two parameters were used to compare the precision of mechanosensory host location between the different temperature treatments with *X. stemmator*, as well as between the two species tested. First, the bias of host location was quantified as the absolute mean deviation of individual insertions from the centre of the host mimic. Second, the scattering of host location was quantified by the variance of the insertion positions of each individual female.

Significant differences in the general responsiveness of the females among the different treatments were analysed separately in both species using χ^2 -tests. The influence of temperature on quantitative insertion activity in *X. stemmator* and in *P. turionellae* was analysed by Kruskal-Wallis-tests due to the nonparametric distributions of these data.

For the comparison between *X. stemmator* and *P. turionellae*, the quantitative insertion activity was evaluated for each temperature treatment by Mann-Whitney *U*-tests.

The temperature influence on the two precision parameters in *X. stemmator* was analysed by analyses of covariance (ANCOVA) with the covariate body weight (standardised). In order to achieve a normally distributed data set, the variance of the insertion positions was log-transformed (natural logarithm). Both precision parameters were compared between the tropical and the temperate species by two-way analyses of covariance (ANCOVA) with the factor species, the factor temperature treatment and covariate body weight (standardised). All analyses were performed with the software SPSS 12.0.1 for Windows.

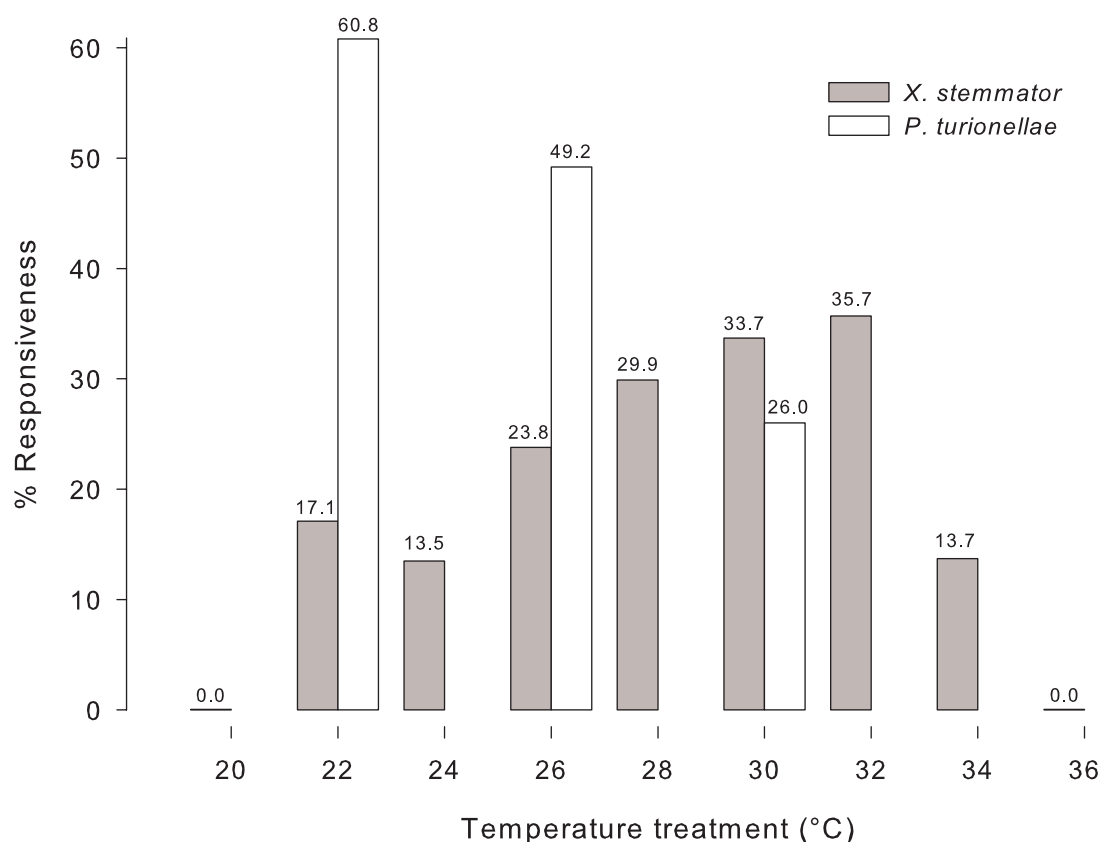


Figure 1. Responsiveness of *X. stemmator* at seven temperature treatments from 22–34°C (grey) and of *P. turionellae* at three temperature treatments at 22°C, 26°C and 30°C (white). Responsiveness is quantified as the percentage of females responding to the plant-stem model with at least one ovipositor insertion.

5.3 Results and discussion

The temperature range allowing for an effective vibrational sounding in the tropical species *X. stemmator* lies between 22 and 34°C, while the use of this mechanosensory host location is not successful at temperatures below 22 and above 34°C (Fig. 1). Over all treatments, *X. stemmator* shows an average proportion of females responding to the host mimic with ovipositor insertions (responsiveness) of 23.9 ± 9.4 % (mean \pm sd). The general responsiveness of the females differ significantly between the temperature treatments (χ^2 -test; $\chi^2=30.9$, $d.f.=6$, $P<0.001$) with the highest value of 35.7 % at 32°C. The responsiveness

declines by more than 20 percentage points with temperature increasing from 32 (35.7 %) to 34°C (13.7 %). By contrast, the temperature range allowing for an effective vibrational sounding in the temperate species *P. turionellae* is broader extending from 8–32°C, with a peak at 18°C, as was found in an associated study (Samietz *et al.*, 2006). To directly compare insertion activity and precision parameters between the two species, a temperature range of 22–30°C was chosen in which both species engage readily in vibrational sounding on the plant-stem model with a hidden host mimic. Even within this relatively narrow range, ambient temperature influenced the responsiveness of the temperate parasitoid *P. turionellae* (χ^2 -test; $\chi^2=25.7$, $d.f.=2$, $P<0.001$) with the lowest temperature tested yielding the largest proportion of responding females with 60.8 % and the highest temperature tested the lowest proportion with 26.0 %, averaging at a responsiveness of 44.5 ± 19.0 % (mean \pm sd) over all temperatures tested (Fig. 1). While the temperate species has been reported to parasitize concealed lepidopteran pupae in orchards already in May and June in Switzerland (Mani *et al.*, 1986), where temperature can easily drop to 8°C and occasionally exceed 30°C, the tropical species fails to engage substantially in vibrational sounding at temperatures below 22°C which are not typical for its habitat (Moore & Kfir, 1996). Furthermore, temperature significantly influences the insertion activity in the tropical (Kruskal-Wallis test; $H_6=22.0$, $P=0.001$) as well as in the temperate species (Kruskal-Wallis test; $H_2=7.57$, $P=0.023$) (Fig.2). The ovipositor insertion activity varies from 1 to a maximum of 41 individual insertions per female in *X. stemmator* and from 1 to 32 individual insertions per female in *P. turionellae*. The lowest activity in *X. stemmator* females (mean \pm s.e.) is at 22°C with 5.17 ± 0.71 insertions and the highest at 32°C with 13.05 ± 1.57 insertions (Tab. 1). By contrast, *P. turionellae* shows the lowest insertion activity at 22°C with 5.00 ± 0.72 insertions and the highest at 26°C with 8.71 ± 1.04 insertions. When comparing the tropical with the temperate species, the mean number of insertions per female differs at 30°C (Mann-Whitney *U*-test; $Z=-2.42$, $P=0.016$), but not at 22 and 26°C (Mann-Whitney *U*-test; 22°C: $Z=-0.102$, $P=0.92$; 26°C: $Z=-0.289$, $P=0.77$). While responsiveness to the host mimic and quantitative insertion activity of *P. turionellae* declines with increasing temperature from 22

to 30°C, activity increases in *X. stemmator* to peak at 32°C although both species were reared under the same conditions during the experiments. The origin of *X. stemmator* is characterized by ambient temperatures around 30°C with no large seasonal variances (Moore & Kfir, 1996). The range of response in our experiment is pivoting around this value in this tropical species and data on ovipositor insertion activity show a clear maximum at these temperatures.

Table 1. Quantitative insertion activity as mean number of insertions (\pm s.e.) per responding female and median, bias of host location quantified as the absolute mean deviation (\pm s.e.) of individual insertions from the centre of the host mimic (expected value at 21.5 section widths) and mean scattering of host location (\pm s.e.) as the variance of insertions' position (in section widths) of *X. stemmator* at seven temperature treatments and of *P. turionellae* at three temperature treatments.

Treatment (°C)	<i>n</i>	Quantitative insertion activity		Bias	Scattering	
		(mean)	(median)			
<i>Xanthopimpla stemmator</i>						
22	30	5.17 \pm 0.71	5.0	1.65 \pm 0.37	7.95 \pm 1.96	
24	20	6.15 \pm 1.38	4.5	2.55 \pm 0.72	10.52 \pm 3.81	
26	30	8.80 \pm 1.30	6.5	1.26 \pm 0.20	11.98 \pm 2.21	
28	20	11.95 \pm 2.22	9.5	1.66 \pm 0.30	9.50 \pm 2.79	
30	30	10.77 \pm 1.52	9.0	1.69 \pm 0.27	13.10 \pm 2.61	
32	20	13.05 \pm 1.57	12.5	2.52 \pm 0.55	20.80 \pm 4.21	
34	20	9.55 \pm 2.26	6.5	1.94 \pm 0.45	15.71 \pm 3.40	
<i>Pimpla turionellae</i>						
22	31	5.00 \pm 0.72	4.0	1.85 \pm 0.34	12.11 \pm 2.84	
26	31	8.71 \pm 1.04	8.0	2.18 \pm 0.34	20.35 \pm 3.45	
30	30	6.87 \pm 1.44	4.0	3.50 \pm 0.73	18.45 \pm 4.99	

Distinct temperature thresholds for vibrational sounding are evident in the precision of the ovipositor insertions. The bias of mechanosensory host location (Fig. 3) differs significantly between the tropical and the temperate species (ANCOVA factor species: $F_{1,182}=8.67$, $P=0.004$). *X. stemmator* females inserted the ovipositor closer to the cue centre than *P. turionellae* females at all three temperatures compared here. Nevertheless, neither the temperature nor body weight has a significant effect on the bias (ANCOVA; factor temperature: $F_{2,182}=2.81$, $P=0.063$; covariate body weight: $F_{1,182}=0.270$, $P=0.60$). The scattering

is not affected by temperature and does not differ significantly between species (ANCOVA; factor species: $F_{1,150}=0.412$, $P=0.52$; factor temperature: $F_{2,150}=1.41$, $P=0.25$). Furthermore, the factors species and temperature show no significant interactions in the two parameters of precision (ANCOVA interaction; bias: $F_{2,182}=1.63$, $P=0.20$; scattering: $F_{2,150}=0.409$, $P=0.67$).

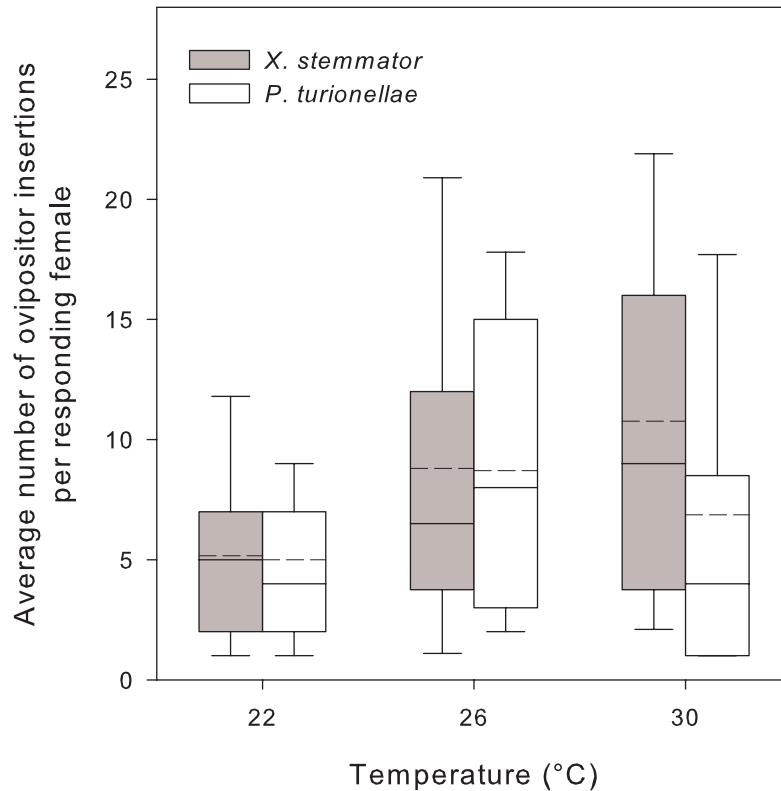


Figure 2. Quantitative insertion activity as mean number of insertions per responding female of *X. stemmator* (grey) and *P. turionellae* (white) at three temperature treatments. Box shows 25th and 75th percentile with median (solid line) and mean (dashed line); error bars show 10th and 90th percentiles.

A remarkable aspect of the results presented here is that the precision of mechanosensory host location can be maintained over a relatively broad range of temperatures. Over all treatments in which females of *X. stemmator* respond positively to the host mimic, there is no significant effect on the bias of mechanosensory host location, i.e. absolute mean deviation of insertions, as well as no interaction between the factor and the covariate (ANCOVA; factor temperature: $F_{6,170}=1.10$, $P=0.37$; covariate body weight: $F_{1,170}=3.47$, $P=0.064$;

interaction: $F_{6,170}=0.705$, $P=0.65$). Also, temperature does not affect the scattering of mechanosensory host location, i.e. variance of ovipositor insertion positions (ANCOVA; factor temperature: $F_{6,141}=0.815$, $P=0.56$; covariate body weight: $F_{1,141}=0.645$, $P=0.58$). Again no interaction between temperature and body weight is found (ANCOVA interaction: $F_{6,141}=1.94$, $P=0.080$). In *X. stemmator*, the highest value (mean \pm s.e.) of bias (2.52 ± 0.55 section widths) as well as the highest value of scattering (20.8 ± 4.2 section widths) are recorded at 32°C (Tab. 1). The lowest values of bias (1.65 ± 0.37 section widths) and scattering (7.95 ± 1.96 section widths) are found at 22°C. The temperate species again shows a broader range of equal precision of vibrational sounding from 10–24°C, but a steep and significant decline below and above those temperatures (Samietz *et al.*, 2006).

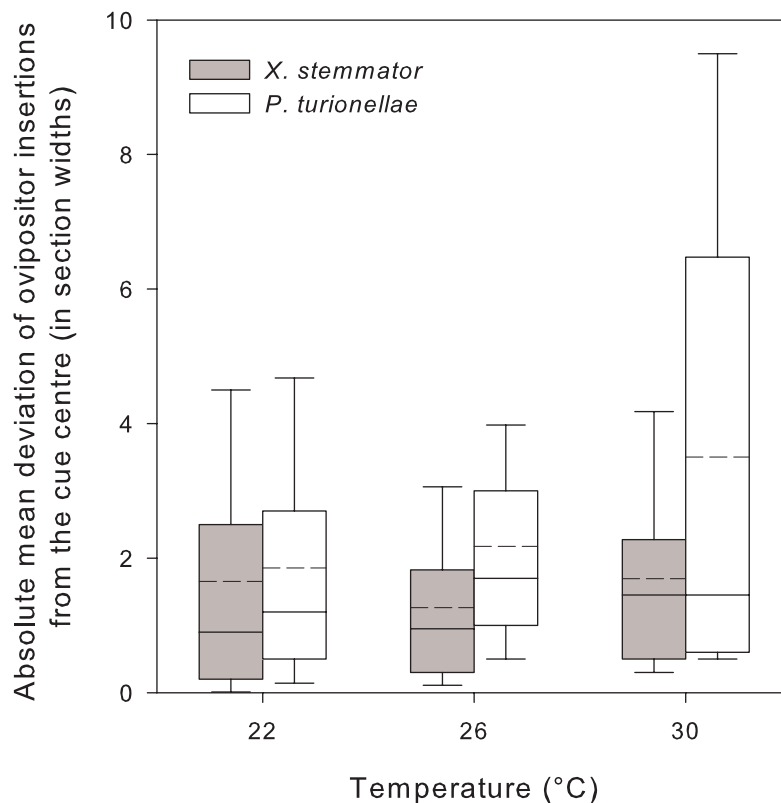


Figure 3. Bias of host location quantified as the absolute mean deviation of individual insertions from the centre of the host mimic (expected value at 21.5 section widths) of *X. stemmator* (grey) and of *P. turionellae* (white) at three temperature treatments. Box shows 25th and 75th percentile with median (solid line) and mean (dashed line); error bars show 10th and 90th percentiles.

By analogy to the evolution of vibrational sounding in two hymenopteran families under a variety of ecological conditions (Broad & Quicke, 2000), it is concluded that this mechanism of mechanosensory host location is adapted strictly to the temperature range encountered in multitrophic species interactions by the parasitoid species concerned.

The precision of the sensory mechanisms studied here depends on the female wasps' ability to produce and receive signals for use in vibrational sounding. Temperature dependence is known in vibration production in spiders (Shimizu & Barth, 1996) and in the physically familiar sound production of crickets (Martin *et al.*, 2000) and cicadas (Fonseca & Revez, 2002). The efficiency of mechanoreceptors as a second part of the sounding system is also influenced by ambient temperature. Many studies report a thermal effect on mechanotransduction, action potential encoding, and action potential conduction in arthropods (e.g. French, 1985; Hoyer & French, 1999; Franz & Ronacher, 2002). However, the pupal parasitoids seem to cope efficiently with the constraints caused by changed temperature. Both species, *P. turionellae* and *X. stemmator*, use vibrational sounding for host location successfully over a broad range of environmental temperatures. The discrepancy between the expected temperature effect on vibration and the observed performance of the wasps might be due to a separate mechanism for the regulation of the body temperature permitting a correct functioning of vibrational sounding. In bees, flight muscle temperature is increased by endothermic shivering performed at low ambient temperatures (Heinrich & Kammer, 1973; Stabentheiner *et al.*, 2003b). A wing fluttering during host location behaviour could also be noticed in females of the two wasp species (S. Kroder, personal observation), suggesting that the wasps use endothermic heat production to maintain a balance of body temperature. Another explanation for the broad thermal range of the sensory mechanism could be a temperature coupling system as known for cricket- and grasshopper acoustic communication. As the chirp rate and syllable rate of the males' song increase with higher temperature, the females' phonotactic preference on the receiving side changes accordingly (Bauer & von Helversen, 1987; Pires & Hoy, 1992).

In conclusions, the performance of mechanosensory host location by vibrational sounding in the two ichneumonid pupal parasitoids studied is markedly influenced by ambient temperature. Optimum temperature ranges for key behavioural parameters differ between the tropical wasp *X. stemmator* and the temperate wasp *P. turionellae* reflecting climatic conditions of their natural habitats. Mechanosensory host location is more effective within or even limited to the temperature ranges that are corresponding to the origin of each species. The tropical wasps are able to maintain the performance of their mechanosensory host location over a broad range of temperatures in which insertion precision is not affected. Further studies will have to investigate how the success of mechanosensory host location is maintained in detail over the broad temperature ranges found in the present study. This could be approached by investigating the vibratory signals in the time and frequency domain under different environmental conditions, and by studying possible mechanisms of behavioural or physiological thermoregulation.

6. Adjustment of vibratory signals to ambient temperature in a host-searching parasitoid

Abstract. Certain ichneumonid parasitoids (Hymenoptera) use self-produced vibrations transmitted on plant substrate, so called vibrational sounding, to locate their immobile concealed pupal hosts. An ambient temperature dependency with higher frequencies and intensities at higher temperatures is postulated because signals are of myogenic origin. Here, we analysed the temperature influence on vibratory signals in the temperate species *Pimpla turionellae* under different thermal conditions using plant-stem models to elicit host searching behaviour. Signals were measured with laser Doppler vibrometry and analysed for time parameters and frequency components applying fast-Fourier transformations (FFT). The results reveal an unexpected effect of ambient temperature on signals produced by the female wasps. While average values of time parameters (pulse trains, pulse train periods, inter pulse duration) were not changed by ambient temperature, the frequency parameters show an inverse thermal effect. Within the temperature range tested (8 to 26°C), decreasing temperature leads to significantly higher frequency and intensity of the self-produced vibrations in the temperate species. This inverse thermal effect can be explained by a temperature-coupled signal production in the frequency domain. Such adaptation to a thermal suboptimal can function as compensation of negative low-temperature effects on the mechanoreceptors by increased muscle activity resulting in more intense and reliable signals. The option of heterothermy to produce signals reliably during vibrational sounding under low temperature is also discussed.

6.1 Introduction

The use of substrate-borne vibratory signals is widespread throughout arthropods as an information source and a communication pathway (e.g. Lewis, 1983; Hill, 2001; Virant-Doberlet & Cokl, 2004). Examples are related to sexual behaviour (Roces & Manrique, 1996), to aggregation and cooperative foraging behaviour (Schneider *et al.*, 1986), as alarm communication (Wood, 1976) and, in the case of bending waves, to the localization of the vibration source (Miles *et al.*, 2001; Barth, 2002). Certain parasitoids locate concealed host insects by host-specific or self-produced vibrations (Meyhöfer *et al.*, 1994; Fischer *et al.*, 2004a), but information to potential hosts on approaching natural enemies can also be vibration mediated (Bacher *et al.*, 1997; Meyhöfer *et al.*, 1997b; Djemai *et al.*, 2001). Vibrations are the least costly signals for communication in arthropods; the range can be up to 1000 times the length of the body, propagation is little diffuse, the signal is confined within the substrate and is therefore easier to locate, and also less likely to attract enemies (Bennet-Clark, 1998; Barth, 2002; Virant-Doberlet & Cokl, 2004).

Nevertheless, little is known about the influence of environmental factors on the sensory physiology of vibratory systems and especially of temperature as one of the most important abiotic factors. Temperature should be especially influential as the physical activity of insects as well as the medium-specific attenuation of vibrational signals, largely depend on thermal conditions (Gogala, 1985; Bennet-Clark, 1998; Greenfield, 2002). In spiders, for example, higher ambient temperatures result in an increasing frequency of vibratory pulses emitted during intraspecific communication (Shimizu & Barth, 1996). The global physiological status of arthropods also basically depends on ambient temperature which again could influence the production of vibrational signals. In the stingless bee *Melipona seminigra*, the temporal pattern of thoracic vibrations is substantially affected by the energetic status of the individual (Hrncir *et al.*, 2004a). Therefore the vibrations may well also depend on ambient temperature, but, to our knowledge, this has not been investigated yet. For the physically similar acoustical signals controlled by the neuromuscular system it is known

that temperature affects the calling song of field crickets (Pires & Hoy, 1992) and cicadas (Sueur & Sanborn, 2003). As body temperature rises, muscle contractions become faster and stronger and cause changes in the song pattern. According to the thermal effect on insect muscles, frequency and intensity of signals increase with increasing temperature and time components become shorter. A similar relationship to temperature is expected for substrate-borne signals.

The pupal parasitoid *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae) uses self-produced vibrations to locate concealed immobile hosts. This so-called vibrational sounding involves the production, transmission and detection of vibrations on solid substrate (Wäckers *et al.*, 1998; Otten *et al.*, 2002). Two biotic factors known to influence the efficiency of vibrational sounding are (1) parasitoid size (Otten *et al.*, 2001) and (2) mechanical resistance of the substrate (Fischer *et al.*, 2003a). In addition to the production of vibrations, it was recently found that the success of this host location mechanism is also well connected to temperature (Kroder *et al.*, 2006; Samietz *et al.*, 2006). These results match the expectations that the quality of the sensory system declines outside an optimum temperature range to which the species is adapted. However, within a remarkably broad range of temperatures there is hardly any influence of ambient conditions on the precision of host location by vibrational sounding which indicates that the wasps are able to compensate for temperature effects either by keeping the signals constant or by a possible temperature-coupling system (Samietz *et al.*, 2006).

The aim of the present study was to test for the hypothesized signal alteration using laser Doppler vibrometry under different temperature treatments. Vibrations produced and transmitted by females were measured on a plant-stem model. With this approach visual and chemical cues could be excluded and the host-searching female had to rely exclusively on vibrational sounding for host location. Signals were investigated in time- and frequency domains to reveal whether and how they are influenced by ambient temperature.

6.2 Material and methods

Parasitoid rearing

A laboratory strain of *Pimpla turionellae* was reared on pupae of *Galleria mellonella* L. (Lepidoptera: Pyralidae). Adult wasps were confined in plexiglass containers (25 x 25 x 25 cm) at 15°C, 70% relative humidity (rh) and a photoperiod of LD 16 : 8 h, fed with honey and water and allowed to mate. Females were exposed three times a week to host pupae for ovipositing and for host-feeding. Parasitized pupae were stored at 24°C, 60% rh and a photoperiod of LD 16 : 8 h until emergence of the adult wasps.

Experimental design

Females of the parasitoid species attempt to insert their ovipositor into various hollow rounded substrates and show the according host-searching behaviour on such substrates. A plant-stem model (length: 70 mm, diameter: 8 mm) made of sketch paper (Schleicher & Schuell, 25 g m⁻², Dueren, Germany) containing a cigarette filter (15 x 8 mm, Gizeh, Gummersbach, Germany) as solid section imitated the hidden host pupa. Plant-stem models have been successfully used in a number of studies on host location in this insect (e.g. Wäckers *et al.*, 1998; Otten *et al.*, 2001; Fischer *et al.*, 2003a).

The experiments were performed with individual parasitoid females. During a trial a single wasp was confined in a small plexiglass box (40 x 25 x 20 mm) placed in a horizontally lying tempering cup (150 x 100 mm) with a plant-stem model reaching through a hole into the cup directly underneath the box. A small slit (1 x 15 mm) in the bottom of the plexiglass box allowed the females to antennate on the plant-stem model without any other interfering contact. The plant-stem model was attached with a thin insect pin (Original Karlsbader "00", Ernst Wirkner, Eschwege Germany) to a laboratory tripod standing outside the tempering cup (Fig. 1) carrying vibrations of very low intensity.

The temperature inside the cup was regulated by a flow thermostat (compact low temperature thermostat RM6, Lauda Dr. R. Wobser, Lauda-Königshofen, Germany) with water diluted glycerine as cooling- and heating fluid

(1 part water and 1 part glycerine). The inside temperature of the cup was checked with a stationary type-T temperature probe (Omega Engineering, Stamford, CT, U.S.A.), located 20 mm behind the plexiglass box. To prevent disturbing vibrations and vibratory noise from the environment, the experiments were set up on an air-buffered, vibration-attenuated table (Photon Control, Cambridge, U.K.).

For controlling the start and the process of antennation, the recordings were supplemented by video observations (Panasonic EV-BL600, 20 ms per image, Germany). The beginning of the vibration recording was indicated on the video sequence by the flashing of a light-emitting diode. In this way the vibration patterns and the corresponding behaviour of the wasps could be traced.

The measurements were performed at four different temperatures (8°C, 14°C, 20°C and 26°C). As female size affects the efficiency of vibrational sounding (Otten *et al.*, 2001), the body weight of each female was measured with a high-precision balance (Mettler Toledo MT 5, Göttingen, Germany; accuracy ± 0.001 mg).

Laser Doppler vibrometry

Vibrations transmitted by the parasitoid during mechanosensory host location on the model were measured with laser Doppler vibrometer (LDV, 2 mW He-Ne-Laser, type: 41 X 62, Dantec, Skovlunde, Denmark) as follows. The laser beam was focused onto a dot of retroreflective tape (Scotchlite, 3M, Neuss, Germany) to obtain an optimal reflection. This tape was attached to the surface of the plant-stem model at a distance of 22 mm from the filter outside the cup.

The output of the LDV is a voltage signal directly proportional to the measured velocity component. In order to digitize the analogue signals the recordings were first cleaned of frequencies over 25 kHz with a low-pass filter (Stanford Research Systems SR650, Sunnyvale, CA, U.S.A.) and subsequently sampled by a data acquisition system on an Apple Macintosh computer (InstruNet Model 100B, GW Instruments, Somerville, MA, U.S.A.) with a sampling rate of 50000 points s^{-1} . Thus, the measurements could be analysed in the frequency domain up to 25 kHz.

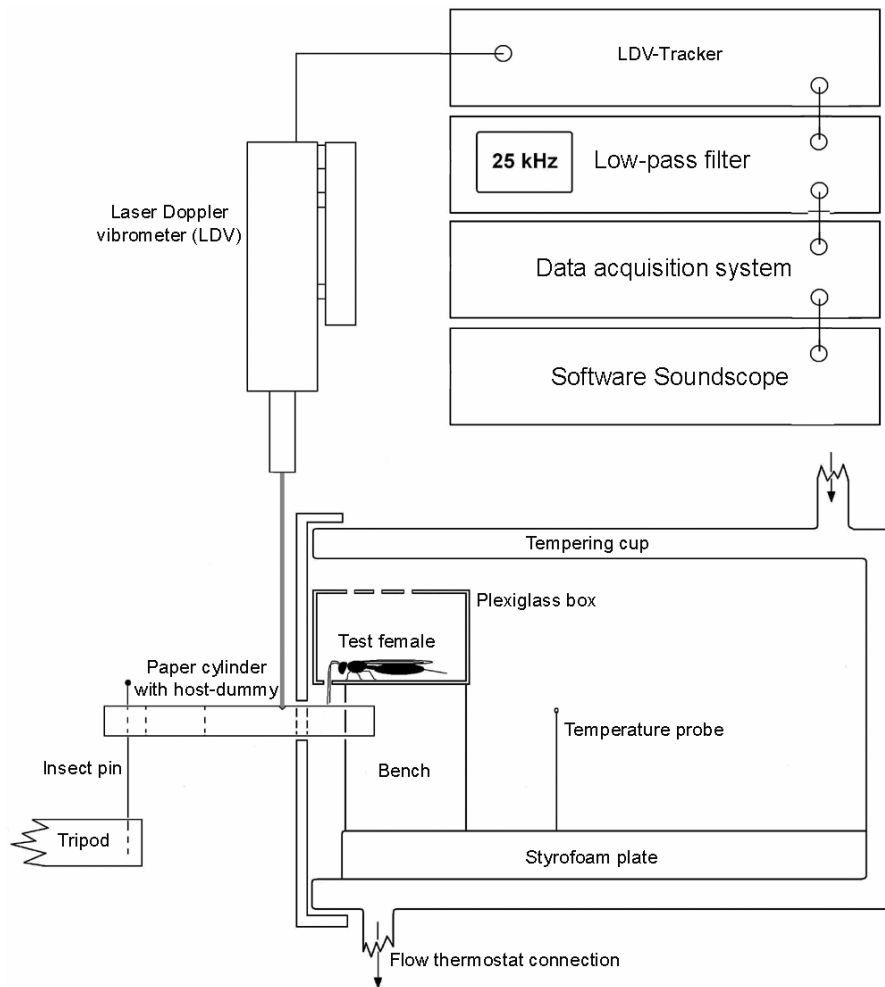


Figure 1. Experimental set-up used to measure vibratory signals of *P. turionellae* females with a laser Doppler vibrometer under different thermal conditions. The laser beam receives the vibrations transmitted by the parasitoid to the paper cylinder. Voltage output (directly proportional to velocity of vibrating particles) is cleaned of frequencies above 25 kHz with a low-pass filter and sampled with a digitizer. The analysis is performed with the software SoundScope.

Signal analysis

The terminology used for the time components of the vibratory signals is according to Meyhöfer *et al.* (1994) and Hrnčir *et al.* (2004a). It is based on the measured velocity of the vibrating substrate (Fig. 2). The females elicit trains of vibrations, composed of pulses, during vibrational sounding. Only peaks in the time series of the vibrations with a velocity of more than 0.2 mm s^{-1} and a

hysteresis of 0.01 can safely be taken to have been generated by the vibrating female and are referred to as single pulses. Consecutive pulses form a pulse train (PT). A pulse train ends when a break of at least 3 ms separates two successive pulses. Such breaks between pulse trains are defined as inter pulse duration (IPD). An upper threshold for IPD in these analyses was set at 200 ms, although the maximum value reached was only 83 ms. Longer gaps are considered to be breaks in the vibrational sounding of the female. The duration from the beginning of a pulse train to the beginning of the next pulse train represents the pulse train period (PTP).

Data were analysed both in the time and frequency domain. The software SoundScope 16 3.0 (GW Instruments, 1993, Somerville, MA, U.S.A.) was used for analysis on an Apple Macintosh computer (G3, 350 MHz). The algorithms contained in SoundScope were programmed to meet the special requirements covered in measuring vibrational sounding (Weinreb & McCabe, 1996). The measurements were high-pass filtered at a threshold of 0.5 kHz to eliminate disturbing low-frequency oscillations. The peaks of pulses served as points of measurements both in the time and frequency domain.

The frequency domain of vibrations was analysed by fast-Fourier transformation (FFT) subdividing the vibrations into frequency components containing a primary oscillation with respective harmonics (Bracewell, 1989). The result of a FFT is a frequency spectrum, referred to as periodogram, i.e. the intensity of the harmonics displayed as a function of the frequency. The frequency with the highest intensity was defined as the carrier frequency. FFT was performed over a range of 5.12 ms (256 points) around each pulse of the time series. Thus, the FFT-result function consisted of 128 points. Subsequently the frequency components and the corresponding intensities of all detected pulses were averaged. A smoothed periodogram between 0 and 25 kHz with a carrier frequency was obtained in each recording. The resolution was 195 Hz corresponding to 128 points extended over 25000 Hz (Fig. 2).

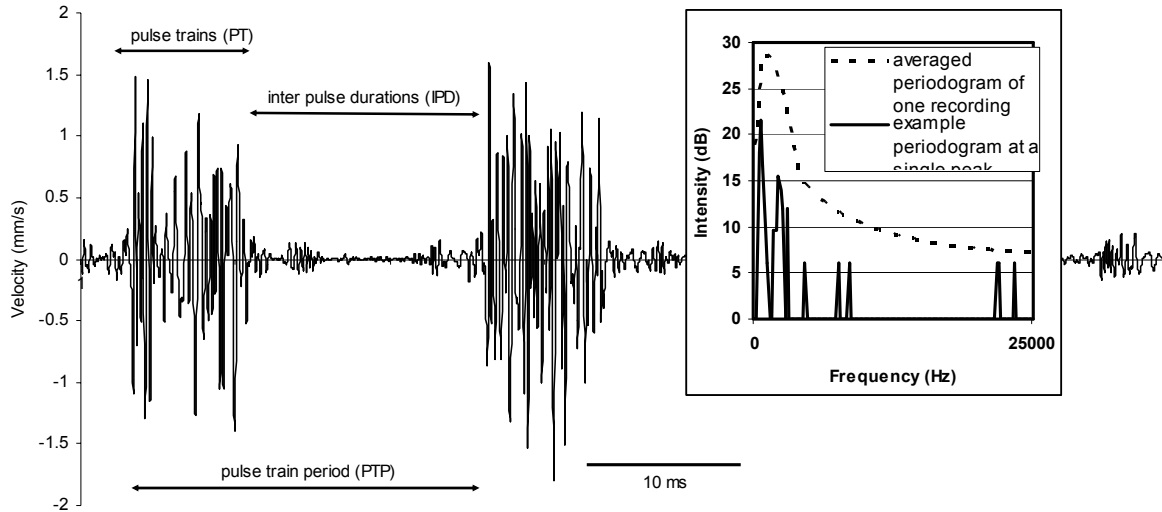


Figure 2. Section from a recording of vibrational sounding in *P. turionellae* at 26°C showing the terminology used for the vibratory signals in a time series, i.e. velocity of vibrating particles as a function of time (large figure). Fast-Fourier transformation (FFT) was conducted at each single pulse resulting in periodograms, i.e. intensity as a function of frequency (inset). The solid line represents an example of a single spectrum calculated around one pulse. The dashed line represents an averaged spectrum over the recording of the example.

The most feasible description of the intensity level (IL) is by definition a logarithmic function with the unit decibel (dB) related to the parameters of acoustic sounds. The formula to compute the IL was

$$IL = 10 \cdot \lg \left(\frac{2}{FFTpoints} \cdot \sqrt{\frac{real^2 + imag^2}{10^{-4}}} \right)$$

In this equation *FFTpoints* is the number of points included in one FFT, *real* is the real point value and *imag* is the imaginary point value of the complex pairs in the FFT resulting wave (Weinreb & McCabe, 1996).

For each single recording, the average duration of PT, PTP and IPD were calculated. Also, the carrier frequency and the intensity at the carrier frequency of the smoothed periodogram were recorded for further analysis of the data.

Data analyses

The vibratory signals of 20 females per temperature treatment were measured. Thus a total number of 80 females in four treatments were tested. The number of

recordings per female depends on individual activity and reaches from 1 to maximal 16, with the following number of measurements made: 96 (8°C), 139 (14°C), 140 (20°C) and 178 (26°C). The recordings taken from single females have durations between 2 and 35 s and comprise a number of peaks between 76 and 19177 with a higher velocity than 0.2 mm s⁻¹ and a hysteresis of 0.01.

The influence of temperature on the frequency parameters of the vibrations (carrier frequency and intensity) were examined using analyses of covariance (ANCOVA) with subsequent multiple *post hoc* comparisons by the Sidak-test. The covariate bodyweight was included in the frequency and intensity analysis as female size is known to influence the intensity of vibrations (Otten *et al.*, 2001). Since the number of records for each individual was different, analyses of covariance were also carried out with mean values weighted by the individual number of records using Weighted Least Squares Regression (WLS). The influence of temperature on the time parameters of the vibratory signals (PT, PTP, IPD) were investigated using analyses of variance (ANOVA). Further, the coefficient of variation of the time parameters was calculated as a dimension of variability and compared for the different temperatures using the procedure of Feltz and Miller (Zar, 1999). The subsequent multiple pairwise comparisons were performed using *Z*-test statistics advanced by Miller (Zar, 1999) with adjusted *P* values by sequential Bonferroni technique (Rice, 1989). All analyses were performed with the software SPSS 12.0.1 for Windows.

Table 1. Frequency parameters of vibrational sounding in *P. turionellae*. Mean values of dominant frequency (Frequ) and intensity at dominant frequency (Int) with standard error of mean (s.e.) at 8, 14, 20 and 26°C. Mean values presented as unweighted data (left) and weighted data by individual number of records using Weighted Least Squares Regression (WLS) (right).

Temperature	Unweighted mean values		WLS - weighted	
	Frequ ± s.e. (Hz)	Int ± s.e. (dB)	Frequ ± s.e. (Hz)	Int ± s.e. (dB)
8°C	1391 ± 28	29.40 ± 0.19	1419 ± 24	29.28 ± 0.16
14°C	1410 ± 30	28.91 ± 0.17	1418 ± 25	29.03 ± 0.15
20°C	1333 ± 30	28.41 ± 0.15	1324 ± 33	28.42 ± 0.17
26°C	1290 ± 21	28.36 ± 0.15	1296 ± 23	28.37 ± 0.16

6.3 Results

Frequency domain of vibratory signals

The carrier frequency of the vibratory signals during vibrational sounding in *P. turionellae* was significantly influenced by environmental temperature but not by body weight (ANCOVA, factor: $F_{3, 79}=4.44$, $P=0.006$, covariate: $F_{1, 79}=1.54$, $P=0.219$) (Fig. 3). The mean values amounted to 1410 Hz at 14°C and 1290 Hz at 26°C (Tab. 1). In the subsequent multiple comparisons significant differences were found between 8 and 26°C, as well as between 14 and 26°C. Signals of a higher frequency were produced at the lower temperatures (*post hoc* Sidak-tests; 8-26°C: $P=0.036$, 14-26°C: $P=0.011$). The effect of ambient temperature on carrier frequency was also significant when the mean values weighted by the individual number of records were used (ANCOVA, WLS weighted mean values, factor: $F_{3, 79}=7.77$, $P<0.001$). Bodyweight did not affect dominant frequency (ANCOVA, WLS weighted mean values, covariate: $F_{1, 79}=3.35$, $P=0.071$). These mean values of carrier frequency amounted to 1419 Hz at 8°C and to 1297 Hz at 26°C. In the subsequent multiple comparisons significant differences were found between 8 and 20°C, 8 and 26°C, 14 and 20°C as well as between 14 and 26°C (*post hoc* Sidak-tests, WLS weighted mean values; 8-20°C: $P=0.036$, 8-26°C: $P=0.003$, 14-20°C: $P=0.024$, 14-26°C: $P=0.001$).

The intensity at the carrier frequency was also influenced by temperature (ANCOVA factor: $F_{3, 79}=5.09$, $P<0.001$) (Fig. 4). There was no significant effect of bodyweight on the intensity (ANCOVA covariate: $F_{1, 79}=1.06$, $P=0.169$). The highest value (mean \pm s.e.) was found at 8°C (29.40 ± 0.19 dB) and the lowest at 26°C (28.36 ± 0.15 dB). Significant differences were found in the subsequent multiple comparisons between 8 and 20°C and between 8 and 26°C (*post hoc* Sidak-tests; 8-20°C: $P<0.001$, 8-26°C: $P<0.001$). In the comparison of the mean values weighted by the individual number of records both the effect of temperature and the effect of bodyweight were significant (ANCOVA, WLS weighted mean values, factor: $F_{3, 79}=10.917$, $P<0.001$, covariate: $F_{1, 79}=5.891$, $P=0.018$). The weighted mean value ranged from 28.37 dB at 26°C to 29.28 dB at 8°C. The differences between 8 and 20°C, 8 and 26°C, 14 and 20°C and between

14 and 26°C were significant in the multiple comparisons (*Post hoc* Sidak-tests, WLS weighted mean values; 8-20°C: $P<0.001$, 8-26°C: $P<0.001$, 14-20°C: $P=0.007$, 14-26°C: $P=0.002$).

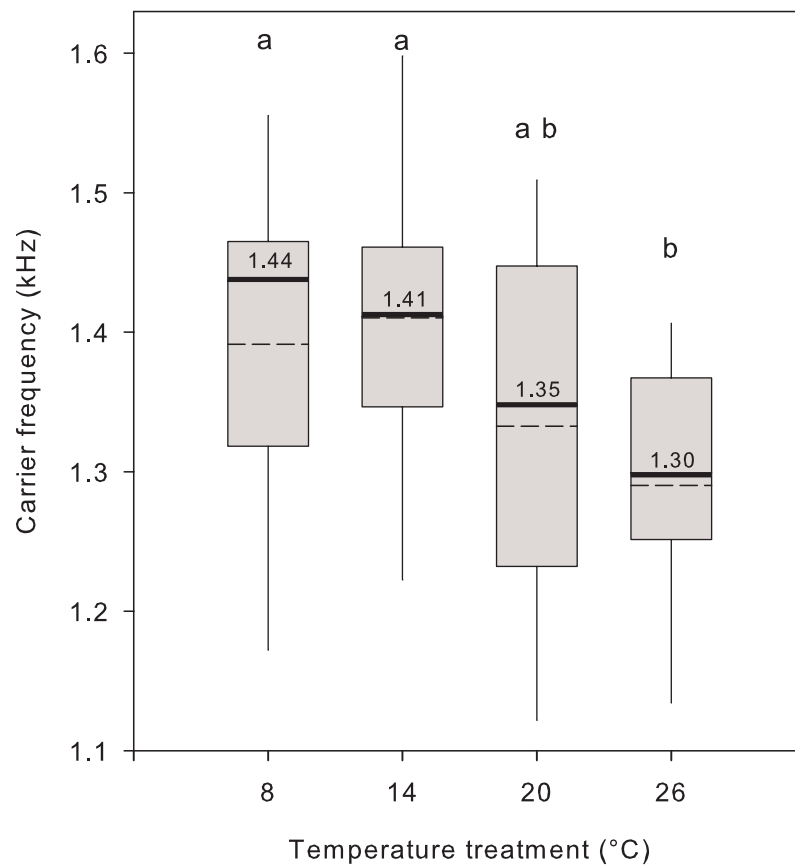


Figure 3. Carrier frequency (unweighted data) of *P. turionellae* at 8, 14, 20 and 26°C. Bold midlines correspond to medians (values shown), dashed lines to means. Boxes range from 25th to 75th percentile. Error lines extend from the 10th to 90th percentile. Significant differences between temperatures are indicated by different letters (ANCOVA, *post hoc* Sidak-test).

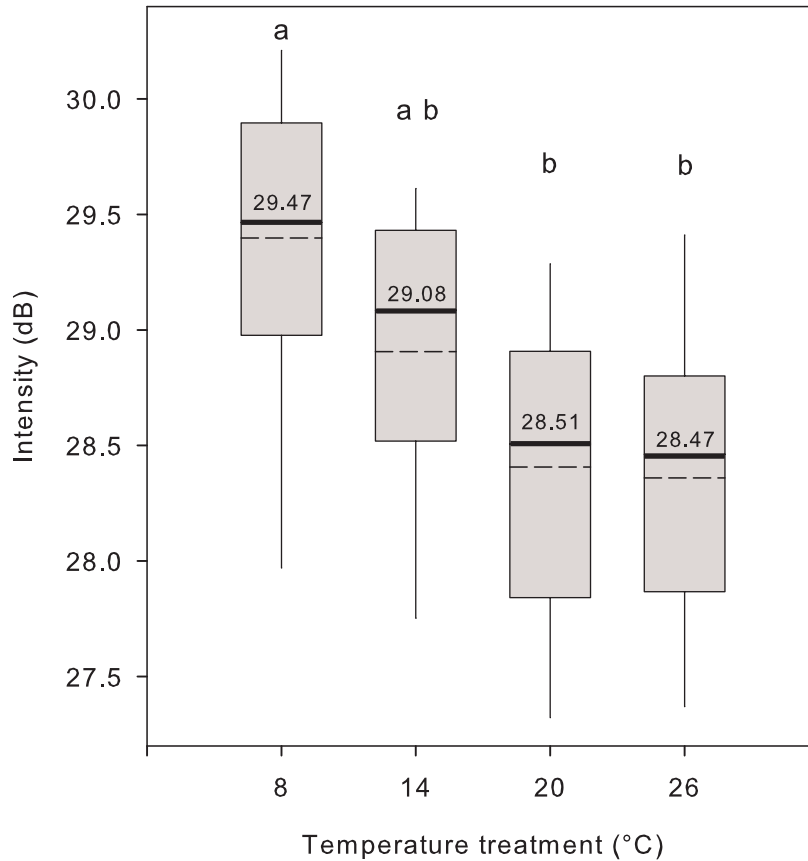


Figure 4. Intensity at carrier frequency (unweighted data) of *P. turionellae* at 8, 14, 20 and 26°C. Bold midlines correspond to medians (values shown), dashed lines to means. Boxes range from 25th to 75th percentile. Error lines extend from the 10th to 90th percentile. Significant differences between temperatures are indicated by different letters (ANCOVA, *post hoc* Sidak-test).

Time domain of vibratory signals

Similar average lengths of the parameters pulse train (PT), pulse train period (PTP) and inter pulse duration (IPD) were detected in all four temperature treatments (Fig. 5). The average length (mean \pm s.e.) of a PT was 8.64 ± 0.85 ms, of a PTP 25.1 ± 2.5 ms and of an IPD 16.4 ± 2.5 ms over all treatments. There was no significant difference between the temperature treatments in these parameters (ANOVA; PT: $F_{3, 79}=1.31$, $P=0.279$; PTP: $F_{3, 79}=0.122$, $P=0.947$; IPD: $F_{3, 79}=0.215$, $P=0.886$). A trend without significance is noticeable in the average duration of a PT, with a maximum at 8°C (9.24 ± 0.52 ms) and a minimum at 26°C (8.22 ± 0.46 ms). By contrast, variability of the parameters characterised by

the coefficient of variation (CV) differed significantly (CV- χ^2 test; PT: $\chi^2=13.2$, $d.f.=3$, 76, $P=0.001$; PTP: $\chi^2=12.5$, $d.f.=3$, 76, $P=0.009$; IDP: $\chi^2=10.9$, $d.f.=3$, 76, $P=0.022$). In pairwise comparisons, both PT and PTP variations differ significantly between all temperatures used in the experiments except of the difference of PT variation between the extreme temperatures of 8 and 26°C in both of which variations were significantly higher than in the intermediate temperatures (CV pairwise-tests; PT, 8-14°C: $P<0.001$; PT, 8-20°C: $P<0.001$; PT, 14-20°C: $P<0.001$; PT, 14-26°C: $P<0.001$; PT, 20-26°C: $P<0.001$; PTP, 8-14°C: $P<0.001$; PTP, 8-20°C: $P<0.001$; PTP, 8-26°C: $P<0.001$; PTP, 14-20°C: $P<0.001$; PTP, 14-26°C: $P=0.002$; PTP, 20-26°C: $P<0.001$). Further, the variability of IPD was significant higher at 8°C than at all other temperatures used in the experiments (CV pairwise-test; IDP, 8-14°C: $P<0.001$; IDP, 8-20°C: $P<0.001$; IDP, 8-26°C: $P<0.001$).

6.4 Discussion

The results of this study show an unexpected effect of ambient temperature on the vibratory signals produced by the female wasps during host location. Although vibratory or acoustic signals in ectotherms are usually slower and less intense at low temperatures, here the time parameters were not changed by ambient temperature for their duration and only affected with respect to their variation. Moreover, the frequency parameters even show an inverse thermal effect during vibrational sounding. Decreasing ambient temperature leads to higher frequency and intensity of the self-produced vibrations.

Reports on vibratory or acoustical signals in other ectotherms document a positive relationship of frequency and intensity to temperature. Higher body temperatures elevate the muscle contraction rates and allow faster and stronger oscillation of the muscles that generate the vibrations and hence produce higher frequencies with a higher intensity (Greenfield, 2002). Such a correlation between temperature and sound power is well known in cicada calling songs where acoustic output and song intensity are highly dependent on body

temperature (Sanborn, 1997; Sueur & Sanborn, 2003). The fact that vibratory signals of *P. turionellae* do not show an according effect indicates an independence of signal production from ambient temperature. Such independence could be achieved by thermoregulation of the body in a temperature range in which signal production is not or negligibly affected. Many poikilotherms and in particular several hymenopteran species raise their body temperature above the environmental temperature by temporary endothermy (Heinrich & Kammer, 1973; Heinrich, 1993; Willmer *et al.*, 2000; Stabentheiner, 2001). This so-called heterothermy (Heinrich, 1993) is exemplified in bumblebees (*Bombus vosnesenskii*) which actually stabilise their thoracic temperature at 33-36°C over ambient temperatures ranging from 5-30°C (Heinrich & Kammer, 1973). Similarly, the cicada *Tibicen winnemanna* is able to maintain tymbal muscle temperature 13°C above ambient temperature by a warm-up buzz previous to full song production (Sanborn, 2001).

Also, none of the time parameters during vibrational sounding in *P. turionellae* is thermally influenced with respect to the average values. This adds further evidence for a regulation of body temperature. Other arthropods show a strong temperature dependence of temporal signal structures. For example, temporal signal patterns of acoustic communication in crickets are strongly linked to temperature (Pires & Hoy, 1992). Likewise, the duration of most signal parameters during vibratory courtship communication of the spider *Cupiennius salei* are thermally affected, though there are also temperature-invariant parameters such as the duty cycle (Shimizu & Barth, 1996). Whereas average values of time parameters in *P. turionellae* were not changed by ambient temperature, their variation changed and was much stronger at extreme than at moderate temperatures. Higher individual variability to deal with sub-optimal conditions might underlie this finding. The wasp population may consist of more or less capable regulators on the extremes. Furthermore, this finding corresponds to the strong thermal dependence of responsiveness, activity and precision of mechanosensory host location in this species (Samietz *et al.*, 2006) which could be also attributed to individuals with different thermoregulatory capabilities.

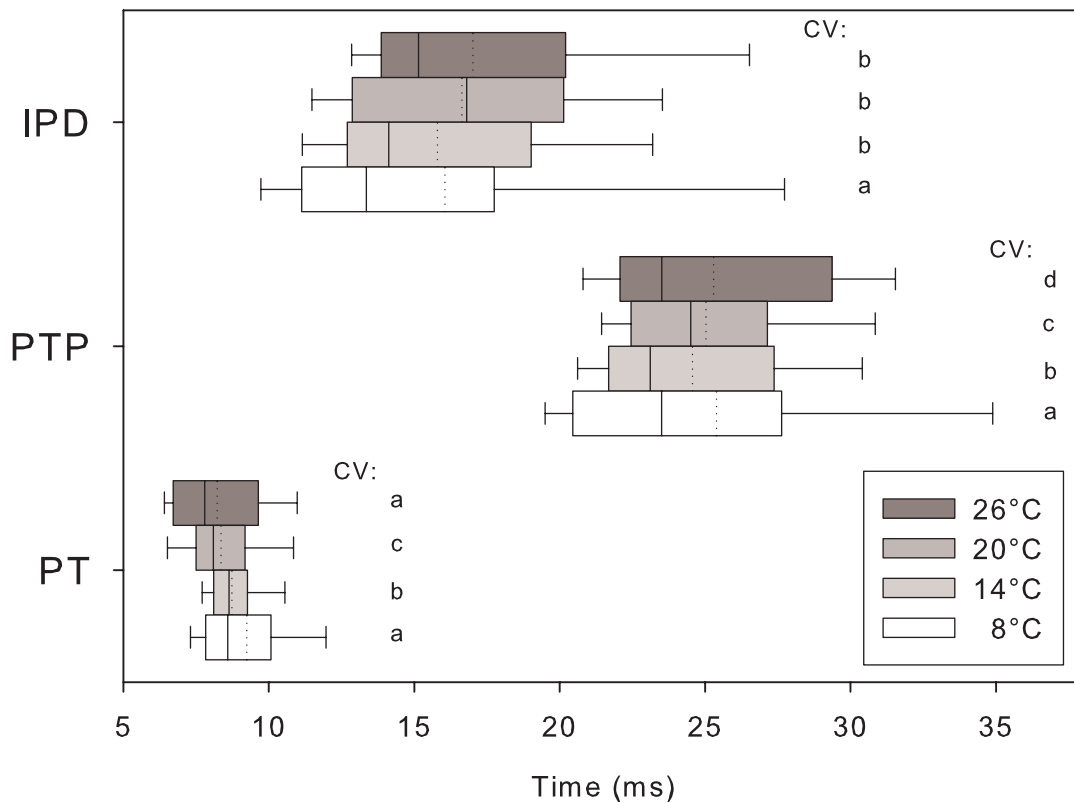


Figure 5. Durations of pulse trains (PT), pulse train periods (PTP) and inter pulse durations (IPD) of *P. turionellae* at 8, 14, 20 and 26°C. Midlines correspond to medians, dashed lines to means. Boxes range from 25th to 75th percentile. Error lines extend from the 10th to 90th percentile. Multiple comparisons (CV) refer to significant different coefficients of variation between temperatures and are indicated by different letters (Pairwise-Z-test with adjusted *P*-value by sequential Bonferroni adjustment).

The question arises why signals are not characterized by a constant high intensity and frequency over a broad range of temperature under a postulated thermoregulation. Whereas the former discussion focuses on the production side of the signals, the observed inverse pattern of carrier frequency and intensity of vibrations in this parasitoid can be related to the receiving side of the sensory system. The subgenual organ by which the host-searching female receives the self-produced vibrations is located in the tibiae (Otten *et al.*, 2002), and the proposed heterothermy would hardly affect the temperature there. In mechanoreceptors of arthropods, however, substantial thermal effects on mechanotransduction, action potential encoding, and action potential conduction

are well known (e.g. French, 1985; Coro & Perez, 1990; Franz & Ronacher, 2002). Decreasing temperature leads to higher response thresholds and a lower sensitivity to physical stimuli (Coro & Perez, 1990; Franz & Ronacher, 2002). Therefore, a higher amplitude of the vibratory signal has a quantitative impact on displacement, velocity and acceleration of the vibration receptor in insects and results in stronger stimulation of the linked sensory nerves (Greenfield, 2002). With higher intensity and frequency of vibrations, *P. turionellae* produces more reliable signals as stronger intensity leads to higher precision of the host searching activity (Otten *et al.*, 2001).

In acoustic communication of crickets, temperature dependent variability in calling song production and accordingly adjusted phonotactic preference of the receiving females is referred to as “temperature coupling” (Pires & Hoy, 1992). In the case of vibrational sounding in *P. turionellae*, the temperature dependent signal production and signal detection would represent a further kind of temperature coupling. The parameters of the self-produced vibrations here are most likely adjusted to the shift of the response threshold in the mechanoreceptors of the parasitoid at low temperatures.

Based on the present results we postulate that the female wasps compensate for ambient temperature changes with their muscle activity to achieve an independence of ambient temperature during vibrational sounding. By means of heterothermy, wasps can control their body temperature and further adjust the vibratory signals to an optimum. The hypothesis of heterothermy during vibrational sounding is supported by the fact that the females are able to elevate vibratory signals at low temperatures. Such increased intensity and frequency result in more reliable signals which balance negative thermal influences on the mechanoreceptors. A temperature-coupled signal production can be considered for such an adjustment of the frequency domain of the vibrations. As a consequence, the wasps would be able to use vibrational sounding successfully in a broad range of ambient temperature as shown in a recent study (Samietz *et al.*, 2006). However, whether or not *P. turionellae* is actually able to maintain and regulate body temperature during vibrational

sounding has to be tested in further experiments including real-time measurements of body temperature during the host-location process.

7. Body temperature of the parasitic wasp *Pimpla turionellae* (L.) (Hym.) during host location by vibrational sounding

Abstract. The pupal parasitoid *Pimpla turionellae* uses self-produced vibrations transmitted on plant substrate, so called vibrational sounding, to locate immobile concealed pupal hosts. Although a temperature dependency of this host location mechanism can be postulated because signals are of myogenic origin, the wasps are able to locate concealed hosts reliably over a broad range of ambient temperatures and even show an inverse thermal effect on frequencies and intensities. The present study investigates how control of body temperature in the wasps by endothermic mechanisms may facilitate host location under changing thermal environments. Insect body temperature was measured with real-time IR thermography on plant-stem models at temperature treatments of 10, 18, 26 and 30°C while behaviour was recorded with respect to vibrational host location. The results reveal a low-level endothermy that likely interferes with vibrational sounding as it only occurs in pausing females. At the lowest temperature of 10°C, thorax temperature was 1.15°C warmer than the ambient surface temperature whereas at the high temperatures of 26 and 30°C the wasps cooled down their thorax by 0.29°C and 0.47°C, respectively, and their head even by 0.45°C and 0.61°C below ambient surface temperature. By contrast, regardless of ambient temperature searching females always had a slightly elevated body temperature of at most 0.30°C above ambient surface temperature. Behavioural observations indicated that searching females more frequently interrupt host location at suboptimal temperatures performing thermoregulation while pausing. For the previously observed inverse thermal effect on frequency and intensity of vibrations, a temperature-coupled production of signal quality can be concluded which compensates for the negative thermal influence on the mechanoreceptors involved.

7.1 Introduction

Nearly all biological processes, rates and functions are influenced by temperature and the smaller an organism is, the more its body temperature is influenced by ambient conditions (Willmer *et al.*, 2000). Insects are therefore to most extent ectotherms and possess physiological and behavioural traits that are well adapted to the temperatures of their habitats (Heinrich, 1993; Willmer *et al.*, 2000; Kroder *et al.*, 2006). Nevertheless, many insects established the capability of maintaining a balance of body temperature by ecto- and endothermic means. Several ectotherms, for example, elevate their body temperature by basking (i.e. absorbing solar radiation) or microhabitat selection but also avoid overheating by selecting cooler sites (e.g. Heinrich, 1993; Kührt *et al.*, 2005; Samietz *et al.*, 2005). In particular several hymenopteran species are able to raise and maintain their body temperature above the environmental temperature without external heat sources (Esch, 1988; Heinrich, 1993; Stabentheiner *et al.*, 2003b). Such a temporary endothermy is referred to as heterothermy and is well studied in honeybees and bumblebees (Heinrich, 1981; Stabentheiner *et al.*, 2003a) but is also found in other insect taxa like Homoptera (Sanborn, 2001) and Lepidoptera (Esch, 1988).

In parasitoids, host location is an essential element of reproduction and accordingly one of the most important processes in the life cycle of these insects. However, the impact of temperature on host location and on the mechanisms involved is largely unknown yet. Mechanosensory mechanisms of host location should be particularly affected by temperature as they depend on physical properties of the environment as well as on the physical activity of the insect (Michelsen *et al.*, 1982; Gogala, 1985; Otten *et al.*, 2001; Cokl & Virant-Doberlet, 2003; Fischer *et al.*, 2003a).

The temperate pupal parasitoid *Pimpla turionellae* (L.) has recently been found to use vibrational sounding successfully in a temperature range from 8 to 28°C (Samietz *et al.*, 2006). Vibrational sounding is a mechanism of mechanosensory host location used in several ichneumonid and orrussid parasitoids of concealed hosts (Wäckers *et al.*, 1998; Broad & Quicke, 2000;

Kroder *et al.*, 2006). Similar to the principles of echolocation, female wasps transmit self-produced vibrations onto the substrate and receive the reflected signals allowing to locate and recognize hidden lepidopteran pupae as their potential hosts (Otten *et al.*, 2002; Fischer *et al.*, 2003a, 2004a). The subgenual organs on the tibiae are known as mechanoreceptors receiving the vibrations (Otten *et al.*, 2002). It is assumed that the vibratory signals originate from muscles in the insect's body and hence should depend on temperature. Behavioural assays confirm that the performance of vibrational sounding drops towards the high and low limits within the range of potential habitat temperatures (Samietz *et al.*, 2006). Although vibratory or acoustic signals in ectotherms are usually slower and less intense at low temperatures, vibration measurements by laser Doppler vibrometry in *P. turionellae* revealed increasing frequency and intensity with decreasing ambient temperature (Kroder *et al.*, submitted).

The observed inverse effect of temperature on vibrations could be caused by temperature-coupled production of signal quality to compensate a negative influence of low temperature on mechanoreceptors. It also indicates a higher muscle activity at low temperatures during vibrational sounding in the female wasps. Accordingly, it is postulated that *P. turionellae* uses temporary endothermy by metabolic heating to perform mechanosensory host location successfully. Here, it should be tested whether the parasitoid females are able to raise or lower their body temperature in relation to ambient thermal conditions and thereby optimize host location by vibrational sounding. Using infrared thermography insect body surface temperatures were measured precisely on the head, thorax and abdomen without any interfering contact. Simultaneously to the temperature measurements, the behaviour of the parasitoids was observed and recorded with respect to general activity and host searching performance.

7.2 Material and methods

Parasitoid rearing

The laboratory strain of *P. turionellae* was reared on pupae of *Galleria mellonella* (Lepidoptera). Adults were confined in Plexiglas containers (25 x 25 x 25 cm) fed with honey and water and were allowed to mate. The wasps were kept at 15°C, 70% relative humidity (rh) and a photoperiod of LD 16 : 8 h. Host pupae were offered three times a week for oviposition and host feeding. Parasitized pupae were stored at 24°C, 60% rh and a photoperiod of LD 16 : 8 h until emergence of adults.

Experimental set-up

The experiments were performed under fluorescent light in a climatic exposure test cabinet (EHRET KLT/04, Emmendingen, Germany) to control ambient temperature and rh of each treatment. The test females of an age between one and four weeks were observed and measured individually in a plexiglass box (196 mm x 88 mm x 80 mm) inside the cabinet. A data logger (Almemo 2590-8, Ahlborn, Holzkirchen, Germany) recorded ambient air temperature and relative humidity in the trial plexiglass box.

The females of the parasitoid innately attempt to insert their ovipositor into various hollow rounded substrates. A paper cylinder (length: 55 mm, diameter: 8 mm) made of airmail paper (ELCO Atlantic Clipper Air Mail, 45 g m⁻², Allschwil, Switzerland) containing a cigarette filter (15 x 8 mm, Gizeh, Gummersbach, Germany) as solid section imitated the hidden host pupa. This experimental approach allowed for offering solely mechanosensory cues excluding any chemical and visual cues. Hence, directed orientation can be attributed to vibrational sounding. Such plant-stem models have been successfully used in a number of studies on host location in these insects (e.g. Wäckers *et al.*, 1998; Fischer *et al.*, 2001; Otten *et al.*, 2001; Fischer *et al.*, 2003a). The model was fixed horizontally on the black back board and black bottom of the box. Because of the rather small mass of the insects (37.5 ± 6.6 mg; overall mean \pm sd) their body temperature was measured thermographically (Stabentheiner & Schmaranzer,

1987). An infrared (IR) thermography camera sensitive at wavelength of 7-12 μm (ThermaCam SC2000 NTS, Flir Systems, Danderyd, Sweden) was directly focused on the parasitoid during the trials with a distance of 20 cm between camera and wasp. The camera transformed the thermal radiation emitted from the insects' surface into electrical impulses, which were amplified and resulted in the so-called thermogrammes, i.e. images showing temperature in shades of grey or in colour steps (Stabentheiner & Schmaranzer, 1987; Stabentheiner *et al.*, 2003b; Stabentheiner *et al.*, 2003a). IR images were stored digitally at a rate of 5 Hz on a DOLCH FlexPac computer (Kontron, Echingen/München, Germany). A self-constructed (Anton Stabentheiner) reference source with a preset temperature of approximately 2°C above ambient temperature was placed 0.5 cm above the right end of the model. The surface temperature of the reference source was measured to the nearest 0.3 °C by the data logger, and by the IR camera. The difference between both measurements was used to calibrate the IR camera and enabled an absolute thermographic measurement accuracy of less than 0.7°C. Differences between body parts and ambient surface temperature were determined with a resolution of 0.1°C. Temperature measurements were done with a self-written Excel (VBA) macro which controlled the IR analysis software (AGEMA Research 2.1, Flir Systems) and extracted temperature and humidity data from the logger files. The surface temperature of the head, thorax and abdomen of each female was measured, as well as the surface temperature at two different points on the paper roll model as ambient surface temperature. Thermographic temperature measurements were calibrated using an IR emissivity of 0.97 of the wasp cuticle (Stabentheiner & Schmaranzer, 1987; Kovac & Stabentheiner, 1999), 0.92 of the paper surface, and 0.91 of the reference source. Temperature analysis was done in searching females at three different points in time after at least 30 s of a certain behaviour, i.e. "antennation" or "probing" (defined below), and on images with pausing females at one point in time after at least 3 min of that behaviour.

The body temperature of *P. turionellae* females was measured in four temperature treatments at 10°C (n = 15), 18°C (n = 17), 26°C (n = 15) and 30°C (n = 15). Due to the fact that metabolic heating capacity depends on body size, the

body weight of each female was measured with a high-precision balance (Mettler AT261 Delta range, Göttingen, Germany, precision 0.01 mg).

Behaviour on plant-stem model

Simultaneously to the IR thermography measurements, the behaviour of the searching wasps was recorded starting upon first contact of the parasitoid with the plant-stem model using the software "The Observer 3.0" (Noldus Information Technology, Wageningen, Netherlands). Observations of host location were divided into five different behavioural states. It was further noted whether or not the wasps fanned their wings as fanning probably corresponds with endothermic muscle shivering. The five behavioural states regarding host location performed by *P. turionellae* were largely analogous to those of the ichneumonid species *Xanthopimpla stemmator* (Thunberg) and therefore defined in accordance to Fischer *et al.* (2004a) as follows:

"Antennation": a pronounced surface antennation of the searching individual. Vibrational sounding is indicated by this behavioural state during which the females transmit pulses via the antennae and receive reflected signals through subgenual organs in their tibiae (Henaut & Guerdoux, 1982; Otten *et al.*, 2001).

"Probing": female bends the abdomen to place the ovipositor tip on the substrate surface while she stands still or walks slowly. This behavioural state often occurs alternately with "antennation" and surface antennation is usually continued during "probing".

"Insertion": the ovipositor is inserted into the plant-stem model. This behavioural response indicates host location (Meyhöfer *et al.*, 1997b; Samietz *et al.*, 2006) and occurs usually subsequent to "probing".

"Cleaning": cleaning of wings or hind legs. This behavioural state interrupts the typical searching behaviour on the plant-stem model, i.e. "antennation", "probing" and "insertion".

"Pausing": no visible activity at all.

The behavioural state "wing fanning" was recorded simultaneously. It appears frequently in slow or high speed during the three behavioural states of searching

as well as during "cleaning", but not during "pausing" as the latter is the state without visible activity. The recordings were carried out for a maximum duration of 10 min but were discontinued sooner if the individual left the model for more than one minute or remained in the behavioural state of "pausing" for more than four minutes.

Data analysis

For statistical comparisons the differences between the insects' temperature of head, thorax and abdomen, and the average ambient surface temperature was calculated at each measuring point (ΔT_H , ΔT_T and ΔT_A , respectively). Since these parameters were all measured thermographically absolute measurement errors were eliminated.

Significant deviation of insect body temperature from the ambient surface temperature was determined by One-sample t-test. Effects of different temperature treatments and body weight on ΔT_H , ΔT_T and ΔT_A of searching and pausing females were tested using analysis of covariance (ANCOVA) with multiple comparisons applying Sidak *post hoc* test. Afterwards, temperatures of searching and pausing females were compared in each temperature treatment by analyses of covariance (ANCOVA).

Regarding the observed searching behaviour on the plant-stem model the transition frequencies between the five behavioural states were counted using a state-lag sequential analysis. The transition probabilities, i.e. transition frequency in relation to the total number of transitions, from "antennation" to "pausing", from "antennation" to "probing" and from "probing" to "antennation" were tested for differences between the temperature treatments using Fisher's exact test with adjusted *P*-values by Hommel method (R Development Core Team, 2005).

The time spent in each behavioural state ("antennation", "probing", "insertion", "pausing" and "cleaning") was divided by the total duration of searching behaviour to test the thermal dependence of performing mechanosensory host location in the parasitoid. These relative durations of searching states were tested between the temperatures using nonparametric

Kruskal-Wallis rank sum test and subsequently compared pairwise by Mann-Whitney- U test with adjusted P -values by Hommel method (R Development Core Team, 2005). The thermal dependence of wing fanning was also tested by comparing the relative duration of this behavioural state between the temperatures using the Kruskal-Wallis rank sum test. Subsequent pairwise comparisons were done using the Mann-Whitney- U test with adjusted P -values by Hommel method.

All statistical analyses were conducted using the statistical computation language R (R Development Core Team, 2005).

7.3 Results

Body temperature of the parasitoids

A total of 62 females were used in the temperature treatments at 10°C, 18°C, 26°C and 30°C, of them 42 females during searching activity and 20 females without any activity (pausing).

The head and thorax temperatures of the pausing wasps were significantly higher than the ambient surface temperature at 10°C and significantly lower at 26°C and 30°C (Fig. 1). The difference between body temperature and ambient surface temperature was not significant at 18°C (mean \pm SEM, One-sample t test; head: $0.33 \pm 0.23^\circ\text{C}$, $t_4=1.11$, $P=0.33$; thorax: $0.59 \pm 0.28^\circ\text{C}$, $t_4=1.38$, $P=0.24$; abdomen: $-0.15 \pm 0.09^\circ\text{C}$, $t_4=-0.984$, $P=0.38$). Similarly, no significant difference was noted between abdomen and ambient surface temperature in all temperature treatments (One-sample t test; 10°C: $t_4=0.606$, $P=0.58$; 26°C: $t_4=-2.45$, $P=0.07$; 30°C: $t_4=1.83$, $P=0.14$). Insect head temperature was on average $0.81 \pm 0.23^\circ\text{C}$, that of the thorax $1.15 \pm 0.28^\circ\text{C}$, above ambient surface temperature in the 10°C treatment. In the higher temperature treatments the average head and thorax temperatures were respectively $0.45 \pm 0.24^\circ\text{C}$ and $0.29 \pm 0.29^\circ\text{C}$ below ambient surface temperature at 26°C, and temperatures of $0.61 \pm 0.23^\circ\text{C}$ and $0.47 \pm 0.28^\circ\text{C}$ below ambient surface temperature at 30°C. The effect of the different environmental temperatures was significant on the head and thorax temperature

of the pausing wasps (ANCOVA, factor; head: $F_{3,20}=7.57$, $P=0.003$; thorax: $F_{3,20}=6.79$, $P=0.004$). In the subsequent multiple comparisons, ΔT_H and ΔT_T at 10°C differed significantly from ΔT_H and ΔT_T at 26°C and 30°C (Sidak *post hoc* tests; $\Delta T_H, 10^\circ\text{C}-26^\circ\text{C}$: $P=0.018$; $\Delta T_H, 10^\circ\text{C}-30^\circ\text{C}$: $P=0.004$; $\Delta T_T, 10^\circ\text{C}-26^\circ\text{C}$: $P=0.025$; $\Delta T_T, 10^\circ\text{C}-30^\circ\text{C}$: $P=0.008$). The abdomen temperature did not differ significantly between the treatments (ANCOVA factor; abdomen: $F_{3,42}=1.15$, $P=0.36$). The body weight of the pausing parasitoids (mean \pm sd: 39.1 \pm 2.5 mg, min: 33.8 mg, max: 43.1 mg, n = 20) did not affect the three body temperatures measured (ANCOVA covariate: NS in all treatments).

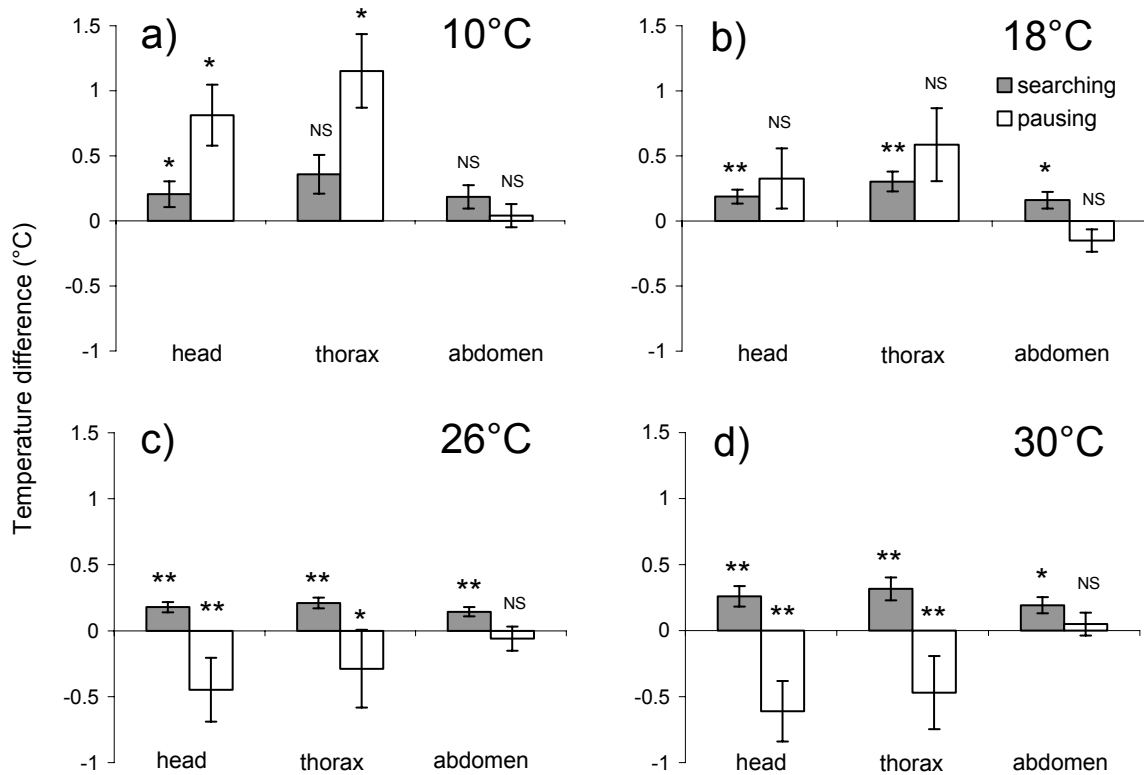


Figure 1. Thermal deviation from ambient surface temperature of head, thorax and abdomen of searching (grey) and pausing females (white) at 10°C (a), 18°C (b) 26°C (c) and 30°C (d). Bars and error whiskers show the mean values and the standard deviations. Significant difference from ambient surface temperature tested by one sample *t*-test (* – $P < 0.05$, ** – $P < 0.01$, NS – not significant).

Table 1. Transition probabilities between behavioural states during host location by vibrational sounding in four temperature treatments, i.e. number of certain transition divided by number of total transitions.

Temperature treatment (°C)	Total time (min)	Total transitions (N)	Antennation to pausing	Antennation to probing	Probing to antennation	Probing to insertion
10	64.4	58	0.172	0.138	0.172	0.000
18	26.6	66	0.015	0.152	0.136	0.030
26	16.3	62	0.065	0.129	0.113	0.016
30	37.2	232	0.086	0.272	0.272	0.022

Searching females showed body temperatures above ambient temperature in all temperature treatments (Fig. 1). Temperatures of all three body parts, i.e. head, thorax and abdomen, were significantly higher at 18, 26 and 30°C. At 10°C, the thorax but not the head and abdomen temperatures were also significantly different from ambient temperature (One sample t-test: head: $t_{9,10}=2.09$, $P=0.066$; abdomen: $t_{9,10}=2.05$, $P=0.070$). Absolute values of differences between female body and environment temperatures were not significantly affected by the temperature level of the environment (ANCOVA, factor: NS in all treatments), nor were they influenced by the body weight of the insects (ANCOVA, covariate: NS in all treatments). Head, thorax and abdomen temperatures of searching females differed from ambient surface temperature by $0.21 \pm 0.07^\circ\text{C}$ (mean \pm s.e.), $0.30 \pm 0.09^\circ\text{C}$ and $0.17 \pm 0.06^\circ\text{C}$ respectively averaged over all four treatments.

As a consequence, head and thorax temperatures differed significantly between searching and pausing females at 10°C, 26°C and 30°C (ANCOVA, factor; 10°C, head: $F_{1,15}=5.35$, $P=0.039$; 10°C, thorax: $F_{1,15}=7.39$, $P=0.019$; 26°C, head: $F_{1,15}=67.9$, $P<0.001$; 26°C, thorax: $F_{1,15}=29.2$, $P<0.001$; 30°C, head: $F_{1,15}=34.1$, $P<0.001$; 30°C, thorax: $F_{1,15}=27.9$, $P<0.001$), and abdomen temperature differed clearly at 26°C (ANCOVA, factor; abdomen: $F_{1,15}=12.4$, $P=0.004$). The significant difference in the abdomen temperature between these different behavioural activities was marginal at 18°C (ANCOVA, factor; abdomen: $F_{1,17}=4.68$, $P=0.048$), whereas head and thorax temperatures between searching and pausing females did not differ at this temperature (ANCOVA, factor; head: $F_{1,17}=0.440$, $P=0.52$; thorax: $F_{1,17}=0.899$, $P=0.36$). No significantly

different abdomen temperatures between searching and pausing wasps were found at 10°C and 30°C (ANCOVA, factor; 10°C: $F_{1,15}=0.030$, $P=0.87$; 30°C: $F_{1,15}=2.41$, $P=0.15$). Body weight of searching females (mean \pm sd: 37.3 ± 6.6 mg, min: 25.9 mg, max: 51.4 mg, $n = 42$) did not affect the three body temperatures measured (ANCOVA, covariate: NS in all treatments).

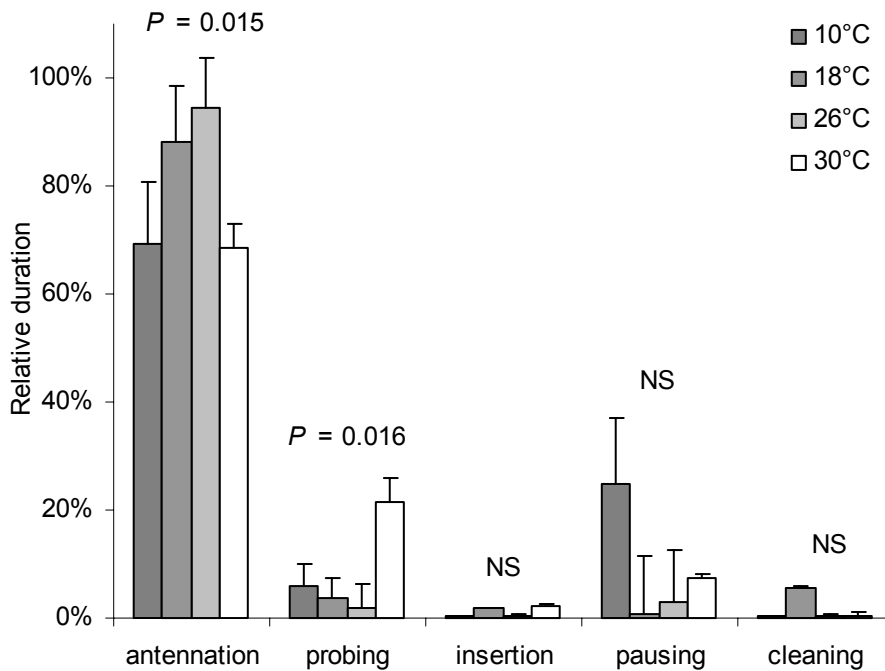


Figure 2. Time fraction during searching behaviour spent with "antennation", "probing", "insertion", "pausing" and "cleaning" as percentages at 10, 18, 26 and 30°C. Bars and error whiskers show the mean values and the standard deviations. Significant temperature influence tested by Kruskal-Wallis rank sum test (P -values, NS – not significant).

Host location behaviour on the plant-stem model

The wasps were observed for a total of 64.4 min at 10°C, 26.6 min at 18°C, 16.3 min at 26°C and 37.2 min at 30°C during host location behaviour (Tab. 1).

Transitions occurred between the behavioural states "antennation" and "probing", "probing" and "insertion", "antennation" and "pausing", "antennation" and "cleaning", and between "pausing" and "cleaning".

Once the parasitoids found the plant-stem model, they immediately started antennating on the model. At the low temperature of 10°C the wasps switched from "antennation" to "pausing" more often than at 18°C (Fisher's Exact Test: $P=0.008$). The transition probability from "antennation" to "pausing" at 26 and 30°C did not differ significantly from the probabilities at other temperatures. The probability of wasps switching from "antennation" to "probing" and backwards from "probing" to "antennation" showed also no significant differences between the temperatures.

The relative duration of "antennation" (Fig. 2) was affected by temperature (Kruskal-Wallis H -test: $H_3=10.5$, $P=0.015$). The highest proportion (mean \pm s.e.) of time spent in this behavioural state was at 26°C (94.4 ± 9.5 %), and the clearly lowest proportion was at 30°C (68.5 ± 4.4 %), difference was only significant between these two temperatures (Mann-Whitney U-test; 26°C-30°C: $W=84.5$, $P=0.041$). Further, the relative duration of "probing" was also affected by temperature (Kruskal-Wallis H -test: $H_3=10.3$, $P=0.016$) and the pairwise comparisons with adjusted p-value by Hommel showed a significantly higher proportion spent in this state at 30 than at 18°C (Mann-Whitney U-test: $W=25.0$, $P=0.0498$). Complementary to "antennation", the highest proportion of searching time in the behavioural state "probing" was spent at 30°C (21.4 ± 4.6 %) and the least was spent at 26°C (1.92 ± 4.39 %) while the relative duration of this state was similarly low at 10 (6.03 ± 4.00 %) and 18°C (3.6 ± 3.82 %). The females barely inserted the ovipositor during the trials, and hence the relative duration of this state was low at 18 (1.7 ± 0.00 %), 26 (0.29 ± 0.00 %) and 30°C (2.05 ± 0.00 %), and did not occur at 10°C. Mean values on relative duration of "pausing" were conspicuously higher at the extreme temperatures of 10 (24.8 ± 12.2 %) and 30°C (7.5 ± 0.6 %), but also varied very much from individual to individual. Temperature treatment did not significantly affect the duration of this state as well as the state of "cleaning" (Kruskal-Wallis H -test; "pausing": $H_3=3.15$, $P=0.37$; "cleaning": $H_3=5.90$, $P=0.12$).

The wasps fanned their wings at all temperatures but the speed of fanning increased with increasing temperature. A large proportion of the time was used for fanning at 26 (93.3 ± 9.2 %) and 30°C (67.4 ± 9.3 %) whereas this activity

covered less than half the time at 10 (21.1 ± 8.78 %) and 18°C (36.0 ± 8.19 %). Furthermore, the variation in the relative duration of this activity was significant between the temperatures (Kruskal-Wallis H -test: $H_3=20.4$, $P<0.001$), and the pairwise comparisons showed differences between 26°C and all other temperatures, as well as between 10 and 30°C (Mann-Whitney U -test; 26°C-10°C: $W=2.0$, $P<0.001$; 26°C-18°C: $W=11.0$, $P=0.003$; 26°C-30°C: $W=83.0$, $P=0.032$; 10°C-30°C: $W=13.0$, $P=0.015$).

7.4 Discussion

The IR-thermography results from host-searching *P. turionellae* wasps revealed a body temperature slightly above the ambient surface temperature, independent of environmental temperature treatments. Females appear to thermoregulate their body while pausing. The body temperature of pausing females, i.e. females without any visible activity, was significantly warmer than the environment at low ambient temperatures and significantly colder than the environment at high ambient temperatures. With respect to host-location behaviour, the wasps paused more often at 10°C whereas they continuously searched at 18°C once they started. Transitions from searching to pausing were also observed at higher ambient temperatures of 26 and 30°C.

The significant gradient between body temperatures and ambient temperature in searching females likely resulted from working muscles. The thorax temperature of host-searching females was generally the highest body part in all treatments. Since muscle activity produces metabolic heat (Heinrich, 1981) this result would confirm the presumed myogenic origin of vibration production in the thorax of the wasps (Henaut, 1990; Otten *et al.*, 2002; Samietz *et al.*, 2006).

In pausing wasps the difference between body and ambient temperature depends on the environmental conditions. In particular the head of the insect is colder than the environment at warm temperatures whereas in particular the thorax is warmer than the environment at cold temperatures. No difference was

found between temperatures of insect body and environment at the moderate temperature of 18°C. Endothermic heating in insects is typically located in the thorax while the head plays a more important role in cooling mechanisms like evaporation by regurgitation (Heinrich, 1981, 1993). These findings suggest a low-level endothermy in *P. turionellae* that probably interferes with vibrational sounding as it only occurs in pausing females. Behavioural observations are in agreement with this latter hypothesis. The searching females interrupt antennation more often at 10°C than at 18°C. In short pausing periods the parasitoids would be able to heat up the body sufficiently to continue with host-location behaviour.

Relatively small levels of endothermic thermoregulation are also reported in other insects, e.g. in *Andrena taraxaci* (Schmaranzer *et al.*, 1997), in the mayfly *Hexagenia bilineata* (Coelho, 1999), and in the beetle genus *Nicrophorus* (Merrick & Smith, 2004). The probability and intensity of endothermy is generally low in small-sized insects like *Pimpla* (Bishop & Armbruster, 1999). Warming up by 1.15°C and cooling down by 0.61°C, as recorded here, are probably not of much physiological influence, but endothermy could be easily combined with ectothermic mechanisms like the absorption of solar radiation (Heinrich, 1981, 1993; Bishop & Armbruster, 1999). In the wasp *Sphecius speciosus* such a combination of behavioural and physiological thermoregulation has already been demonstrated whereby the thorax temperature regulation mainly depends on behavioural mechanisms (Coelho, 2001). Under natural conditions *P. turionellae* would be similarly able to thermoregulate the body by habitat selection or basking as the dark body would be highly absorptive with respect to solar radiation (Willmer & Unwin, 1981). These endothermic mechanisms could then be used in addition to thermoregulate additionally under sub-optimal conditions, e.g. low ambient temperature or low solar radiation. This assumption is supported by the declining difference between thorax and ambient temperature with increasing environmental temperature. The energy investment for endothermy in small animals is very high, and therefore they should reduce physiological heat production with increasing ambient temperature. Likewise, the wasp *Paravespula germanica* – an insect capable of intense endothermy

(Heinrich, 1984; Kovac & Stabentheiner, 1999) – adjusts endothermic heat production to solar radiation and decreases its own energy investment with increasing solar radiation (Stabentheiner *et al.*, 2004).

Accessory wing movement in insects is often linked to 'shivering' as heating mechanism, i.e. endothermic heat production with flight muscles (Heinrich, 1981). The accessory wing movement in *P. turionellae* is, however, probably not linked to heat production as the movement increases at high and not at low temperatures. Accordingly the increasing wing activity could be possibly linked to 'fanning', resulting in a higher convective heat loss. Fanning is used in social insects for ventilation of the nest at high temperature conditions (Heinrich, 1993), but is not known yet to be used as a cooling mechanism for an insect's own body.

A further possible cooling mechanism is by means of increased evaporation of the body which occurs in many hymenopterans and other insects to avoid overheating (Heinrich, 1993). Bumblebees and honeybees, for example, repeatedly regurgitate a droplet of fluid followed by a decrease in head temperature (Heinrich, 1981). A likewise mechanism could be also considered in *P. turionellae* as body temperature was below ambient temperature at high ambient temperature and especially the head showed the lowest temperature.

In conclusion, the parasitoid females studied here are indeed able to raise or lower their body temperature in relation to ambient thermal conditions. Under conditions below or above an optimal temperature range, the host-searching females frequently interrupt the searching process as they are not able to perform vibrational sounding and endothermy simultaneously. Thermoregulation during host searching is, however, far from keeping a constant body temperature over wide ambient temperature ranges and the parasitoids seem to cope very well with high thermal changes during the use of vibrational sounding as the frequency and the intensity of self-produced vibrations actually increase with decreasing temperature (Samietz *et al.*, 2006; Kroder *et al.*, submitted). This inverse effect of temperature on vibrational signals in the wasps can therefore not be explained solely by endothermic heat production combined with behavioural mechanisms of thermal response. It is rather that the wasps

optimize host location by vibrational sounding with temperature-coupled production of signal quality to compensate a negative influence of low temperature on mechanoreceptors.

8. Temperature affects interaction of visual and vibrational cues in parasitoid host location

Abstract. Parasitoid host location in nature is facilitated by simultaneously using different information sources. How multisensory orientation on the same spatial scale is influenced by environmental conditions is however unknown yet. Here we test whether non-proportional changes in reliability of cues can cause parasitoids to alter multisensory orientation and to switch to more reliable cues under extreme temperatures. In the ichneumonid wasp *Pimpla turionellae*, multisensory use of thermally insensitive vision and thermally sensitive mechanosensory host location by vibrational sounding (echolocation on solid substrate) was investigated with choice experiments on plant-stem models under optimum temperature (18°C), at high (28°C) and low temperature limits (8°C) of vibrational sounding. Temperature affects relative importance of vibrational sounding whereas visual orientation did not vary. At 18°C, parasitoids used visual and vibrational cues with comparable relative importance. At 8 and 28°C, the role of vibrational sounding in multisensory orientation significantly depreciated in line with decreased reliability. Wasps nearly exclusively chose for visual cues at 8°C. The parasitoids switch between cues and sensory systems in dependence of temperature. As overall precision of ovipositor insertions was not affected by temperature, the parasitoids fully compensate the loss of one cue provided another reliable cue is available on the same spatial scale.

8.1 Introduction

Host location is a key process for trophic interactions between parasitoids and their hosts in which both sides evolve strategies in response to each other. While hosts evolve strategies to avoid detection (Vet *et al.*, 1991; Vinson, 1998), the

parasitoids may refine their sensory systems or increase the number of information sources to locate and recognize hosts. Latter may involve plant-derived and host-derived signals that parasitoids perceive using sensory systems for olfactory, contact chemical, visual, or mechanosensory cues (for review see Godfray, 1994). The relative importance of cues can vary with their accessibility and reliability during the host searching process (Wellings, 1993; Völkl, 2000; Schwörer & Völkl, 2001). Whereas the accessibility is mainly affected by the spatial scale, the reliability can largely depend on environmental factors. Simultaneous use of cues improves reliability and success of host location (Wäckers & Lewis, 1994; Battaglia *et al.*, 2000; Fischer *et al.*, 2001; Stireman). Although in nature different information sources are available at the same time and allow for multisensory orientation, the vast majority of studies on parasitoid host location is focused on a single cue only (e.g. Michaud & Mackauer, 1994; Wäckers *et al.*, 1998; Dutton *et al.*, 2000; Rott *et al.*, 2005). Some studies have so far investigated the interaction of visual and chemical cues in the same stage of host searching (e.g. Battaglia *et al.*, 1993; Wäckers & Lewis, 1994; Battaglia *et al.*, 2000; Morehead & Feener, 2000; Stireman, 2002). More likely many, or even all host location processes are based on multisensory orientation including several cues which the parasitoids are able to combine in dependence of habitat and environmental conditions. However, whether and how parasitoids are able to switch between cues and sensory systems on the same spatial scale in dependence of environmental factors influencing reliability is hitherto unknown. During host location, the parasitoid *Pimpla turionellae* (L.) (Hym.: Ichneumonidae) is able to trace endophytic lepidopteran pupae by visual orientation (Fischer *et al.*, 2003b, 2004b) as well as by vibrational sounding (Wäckers *et al.*, 1998; Fischer *et al.*, 2003a). Latter is a mechanosensory mechanisms in which female wasps transmit self-produced vibrations on plant material (echolocation on solid substrate) and locate immobile hidden hosts by signal modifications sensed with the subgenual organs (Otten *et al.*, 2002). This mechanism is especially established in pupal parasitoids of endophytic hosts where other cues are quantitatively reduced or not reliable enough (Broad & Quicke, 2000). Visual orientation for potential endophytic hosts can, for example,

be based on tunneling damage, lesions or entrance holes (Pfannenstiel *et al.*, 1992; Smith *et al.*, 1993; Potting *et al.*, 1997).

Studied under moderate ambient temperatures, *P. turionellae* females use both visual and mechanosensory cues simultaneously for short-range host location and the two senses interact, resulting in an additive accuracy (Fischer *et al.*, 2001). When the two cues are offered separately on artificial plant stem models, neither the visual nor the mechanosensory cue is favoured (Fischer *et al.*, 2001). Both are used in this species and seem to be equally important for reliable host location on the investigated spatial scale. However, reliability of vibrational cues may change with changing environmental temperature since the performance and precision of vibrational sounding is obviously negatively influenced by suboptimal, especially too low ambient temperatures of 10°C or less (Kroder *et al.*, 2006; Samietz *et al.*, 2006). In insect vision, however, such negative temperature effects are unknown. Non-proportional changes in reliability of two relevant stimuli could cause the wasps to alter their multisensory orientation and to switch to the more reliable cue under extreme environmental conditions.

The present study tests this hypothesis with choice experiments under optimum temperature, as well as at the high and low temperature limits of vibrational sounding in the parasitoid *P. turionellae*. Furthermore it is tested, if switching to a single available cue under extreme conditions influences the overall precision of host location. In our experiments, plant-stem models containing visual and mechanosensory information – but no chemical cues – are offered to single females and subsequently scored for quantity, location and precision of ovipositor insertions.

8.2 Material and methods

Parasitoid rearing

Laboratory strain of *P. turionellae* was reared on pupae of *Galleria mellonella* (Lepidoptera). Adults were confined in Plexiglas containers (25 x 25 x 25 cm) fed

with honey and water. The wasps were kept at 15°C, 70% relative humidity (rh) with a photoperiod of 16D:8L. The host pupae were offered three times a week for ovipositing and host feeding. Parasitized pupae were stored at 24°C, 60% rh and a photoperiod of 16D:8L until emergence of adults. The females were allowed to mate prior to the experiments and had host contact for the first time 24 to 48 hours before the experiments started.

Experimental set-up

Plant stems with insect tunnels are potential lairs of host pupae, hence, any hollow rounded substrate elicits searching behaviour in *P. turionellae* with subsequent ovipositor insertions. In the experiments of the present study, a paper-cylinder (length: 125 mm, diameter: 8 mm) made of airmail paper (ELCO Atlantic Clipper Air Mail, 45 g m⁻², Allschwil, Switzerland) with both ends left open mimicked a hollow plant stem. A cigarette filter (15 x 8 mm, Gizeh, Gummersbach, Germany) as solid section imitated the hidden host pupa and a black band as visual cue imitated damages by a potential host (Fig. 1). The black band was printed on the airmail paper with a Lanier copier 7335 (Lanier, Horgen, Switzerland; output: 1200 dpi). This experimental approach excluded chemical cues and allowed the parasitoids to use only vibrational and visual cue during host location. The ovipositor insertions on such a plant-stem model can be attributed to vibrational sounding and visual orientation.

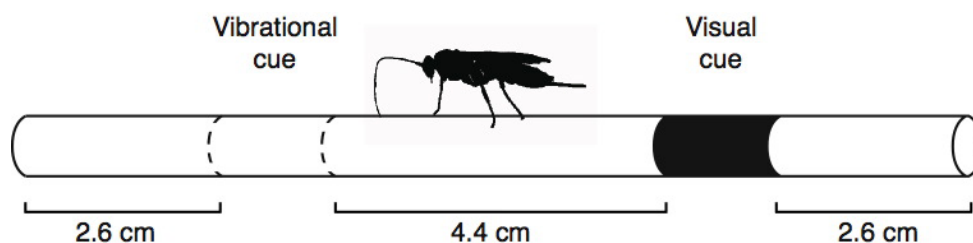


Figure 1. Plant-stem model with vibrational (cigarette filter; width = 1.5 cm) and visual cue (black band; width = 1.5 cm).

The plant-stem models with the choice of vibrational and visual cue were offered to parasitoid females in three temperature treatments at 8, 18 and 28°C. The experiments were conducted in a climate chamber under controlled conditions at test temperature, 60 % rh and white fluorescent illumination. At least one hour before the trials, the parasitoids (age 6 to 33 days) were acclimatized to the test conditions. During the trials, each female was individually exposed to a plant-stem model (Fig. 1) for 20 minutes in a plexiglass box (18.5 x 8.5 x 7.5 cm). At the beginning of the trial, each female was put at a random position within the box. The plant-stem models were horizontally fixed on white bottom of the box. Each individual and plant-stem model was only used once so that the test females were associated to their plant-stem models for the subsequent analysis. This experimental approach has been successfully used in a number of studies on host location in this insect (e.g. Fischer *et al.*, 2001; Kroder *et al.*, 2006; Samietz *et al.*, 2006).

Data analysis

The ovipositor insertions of the parasitoids on the plant-stem models showed how the females use multisensory orientation during host location under changing temperatures. The plant-stem models were subdivided into 34 sections with section widths of 3.7 mm. The cigarette filter as the vibrational cue and the black band as the visual cue contained four sections each. The cues were symmetrically located with a distance of seven sections to the outer ends of the plant-stem model and twelve sections between the cues. This allowed scoring the position of the insertions relative to the two different cues on the plant-stem model. As detailed below, the insertions were quantitatively and qualitatively analysed for overall, vibrational and visual responsiveness, for relative importance of vibrational and visual cue during host location in each treatment, for precision and for insertion activity expressed as the average number of insertions per responding female.

The proportion of females responding with at least one ovipositor insertion to the plant-stem model was defined as overall responsiveness, and the proportions of females responding with insertions on the two cue areas were

defined as vibrational- and visual responsiveness, respectively. Temperature dependence of responsiveness was tested by Fischer's probability test with adjusted P -values by the Hommel method (R Development Core Team, 2005). Furthermore, visual and vibrational responsiveness were compared within each temperature treatment using nonparametric McNemar test. The numbers of insertions on cue areas were divided by the total number of insertions in each female revealing the relative importance of the cues during host location. To avoid disproportionately weighted insertions and to use females spending the majority of trial time in searching, only females with at least three insertions were valid for the comparisons of the relative importance. The desired sample size in each temperature treatment was therefore 20 females with at least three ovipositor insertions. The use of vibrational and visual cue were analysed for temperature dependence applying the nonparametric Kruskal-Wallis H-test. Within each temperature, the preference for one of the cues was tested using the nonparametric Wilcoxon-test. Furthermore, the precision was measured in analogous way to the bias of insertions in Kroder *et al.* (2006) and Samietz *et al.* (2006) by the average deviation from the nearest cue centre. Significant temperature effects on the precision and on insertion activity were tested using the nonparametric Kruskal-Wallis H-test.

All statistical analyses were conducted by the statistical computation language R (R Development Core Team, 2005).

8.3 Results

Responsiveness

A total of 468 females were tested until the desired sample sizes were obtained. The highest overall responsiveness, i.e. the proportion of females inserting ovipositor at least once, with 55.7 % was found at the medium temperature of 18°C (Fig. 2), whereas responsiveness declined significantly to 19.0 % at 8°C and 27.4 % at 28°C (Fisher's test; 8-18°C: $P < 0.001$; 18-28°C: $P = 0.031$). No significant

difference was found between the overall responsiveness at the two extreme temperatures of 8°C and 28°C (Fisher's test: $P=0.152$).

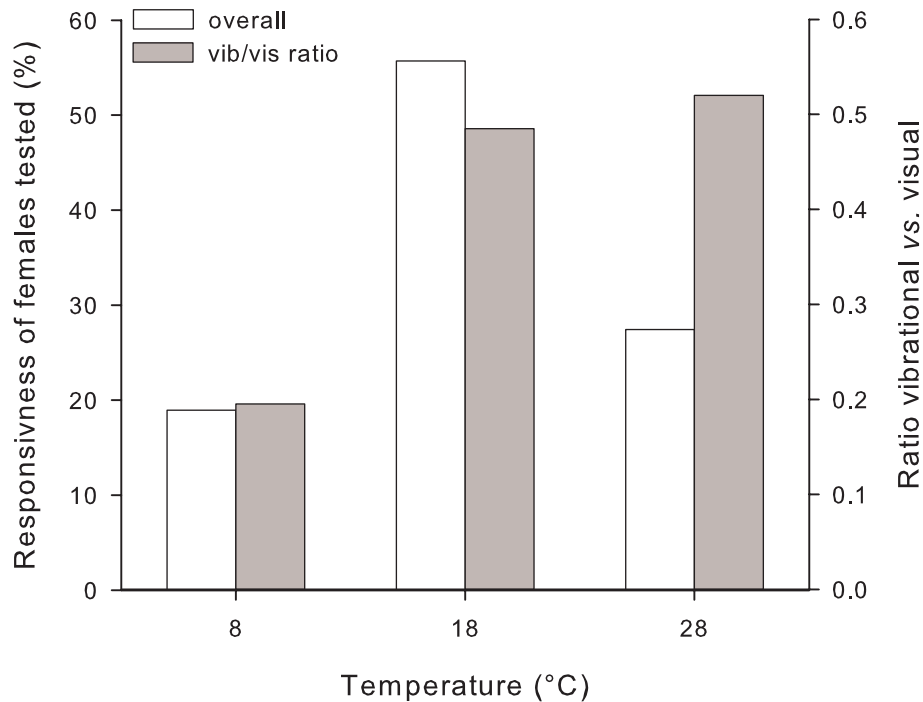


Figure 2. Percentage of overall responsiveness (white; left y-axis) and ratio of vibrational versus visual responsiveness (grey; right y-axis) in *Pimpla turionellae* at 8, 18 and 28°C. Responsiveness is quantified as the females responding with at least one ovipositor insertion.

The responsiveness to the vibrational cue was generally lower than to the visual cue. Significant differences between vibrational and visual responsiveness were found in each temperature treatment (McNemar test; 8°C: $\chi^2=20.9$, $P>0.001$; 18°C: $P=0.001$; 28°C: $P=0.017$). Vibrational responsiveness was lowest at 8°C with only 2.81 % responding females (Fisher's exact probability test; 8-18°C: $P<0.001$; 8-28°C: $P=0.005$). It did not differ between 18 and 28°C despite the relatively different percentages of 22.9 % and 11.5 %, respectively (Fisher's exact probability test: $P=0.104$). By contrast, the visual responsiveness reflected a similar temperature dependence as the overall responsiveness, culminating with the significantly highest proportion of 47.1 % at 18°C (Fisher's exact probability

test; 8-18°C: $P < 0.001$; 18-28°C: $P = 0.030$), and declining with decreasing and increasing temperature to 14.4 % at 8°C, and to 22.1 % at 28°C, without a significant difference between these extreme temperatures (Fisher's exact probability test: $P = 0.145$).

Relative importance of cues

The two cues on the plant-stem model showed a strong influence on host-location behaviour of the female wasps (Fig. 3). Overall 79.7 % of ovipositor insertions were observed directly on the two cue areas, surpassing the insertions on the remaining area significantly in all temperature treatments (Wilcoxon-test; 8°C: $Z = -4.70$, $P < 0.001$; 18°C: $Z = -5.13$, $P < 0.001$; 28°C: $Z = -4.00$, $P < 0.001$).

The quantitative ovipositor insertion activities on two cues offered on the plant-stem model were differently affected by temperature (Fig. 4). Focussing on the females with at least three insertions, a significant temperature effect was noted on the relative importance of the vibrational cue (Kruskal-Wallis H-test: $H_2 = 10.339$, $P = 0.006$) but not on the visual cue (Kruskal-Wallis H-test: $H_2 = 4.025$, $P = 0.134$). At the low temperature of 8°C, the relative importance of vibrational sounding nearly disappeared. At the high temperature of 28°C it was also significantly lower than at 18°C (Fig.2). By contrast, the highest value of the relative importance of the visual cue was found at 8°C, and the use of the two cues differed significantly at this temperature (Wilcoxon-test: $Z = -3.15$, $P = 0.002$). There was also a significant difference between the relative importance of vibrational and visual cue at the highest tested temperature at 28°C (Wilcoxon-test: $Z = -2.08$, $P = 0.037$). At the medium temperature of 18°C both cues, vibrational and visual orientation, were equally used during host location by female wasps. No significant difference was found between the relative importance of visual and vibrational cue (Wilcoxon-test: $Z = -1.17$, $P = 0.241$).

Overall precision and insertion activity

The overall precision of the parasitoids host location was not affected by ambient temperature (Kruskal-Wallis H-test: $H_2 = 1.21$, $P = 0.55$). They inserted their ovipositor with an average deviation (mean \pm *sd*) from the cue centres of $1.26 \pm$

0.96 section widths in total (Fig. 3). The precision values of individuals varied from 0.5 to 6.5 sections deviation from the next cue centre.

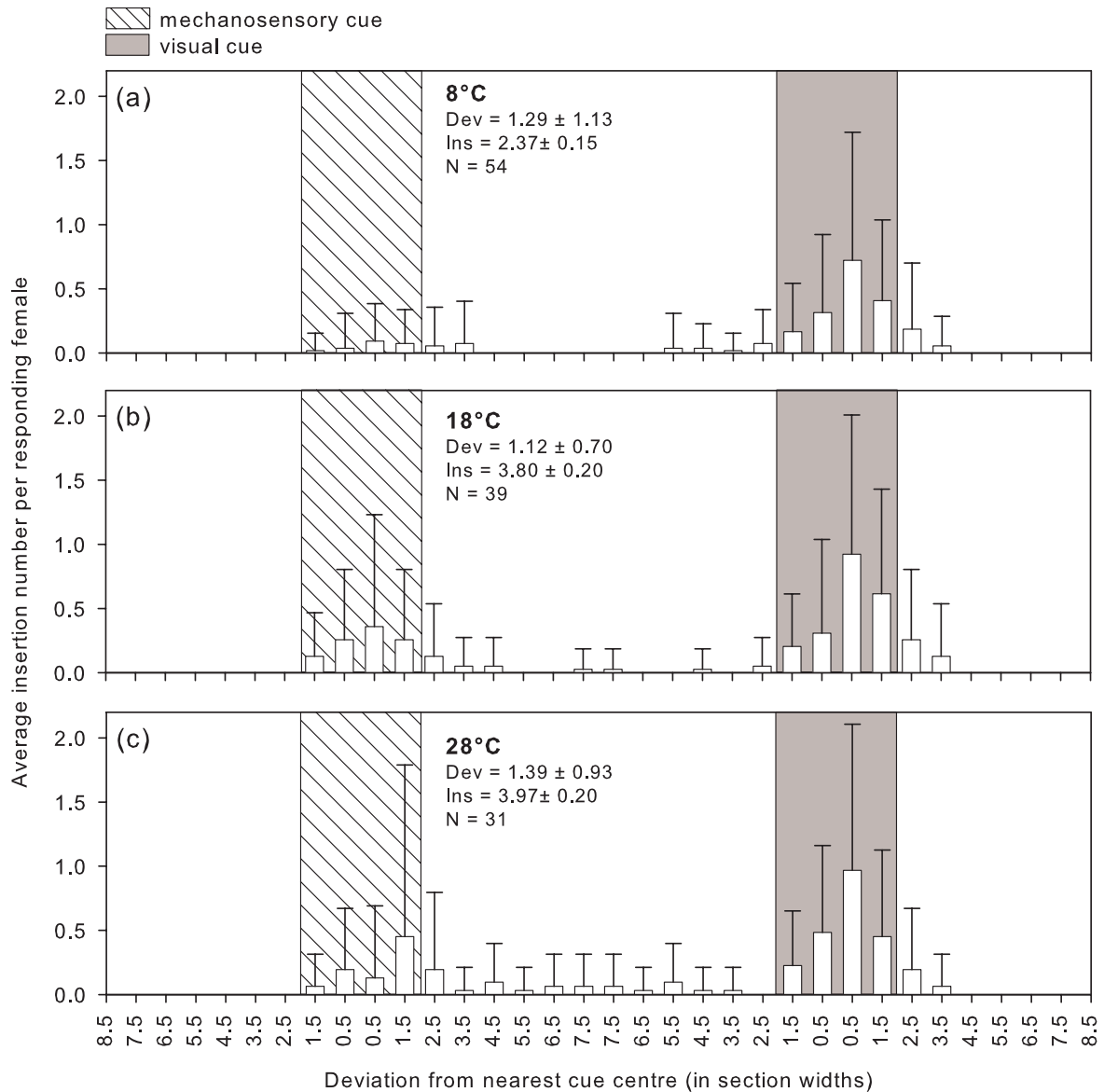


Figure 3. Distribution of ovipositor insertions of *Pimpla turionellae* on plant-stem model with vibrational (cigarette filter) and visual cue (black band) at 8 (a), 18 (b) and 28°C (c). The bars and error bars show average number of insertions per responding female in each section (mean ± *sd*). The parameters are the mean deviation from the nearest cue centre in section widths (Dev; mean ± *sd*) and average number of insertions into plant-stem model per responding female (Ins; mean ± *sd*).

The insertion activity as the average number of insertions per responding female largely depended on ambient temperature (Kruskall-Wallis H-test: $H_2=6.78$, $P=0.034$). Whereas parasitoids rarely inserted the ovipositor into the plant-stem model under the cold condition at 8°C, they increased the insertion activity with increasing temperature (Fig. 3).

8.4 Discussion

The present study provides clear evidence that parasitoids are able to combine different information sources in multisensory host location depending on the environmental conditions. They can switch between sensory systems when the reliability of one host location cue on the same spatial scale is altered due to ambient factors. The insect species studied here switches between an interactive combined use of mechanosensory and visual cues to a nearly exclusive use of visual cues at low temperatures when vibrational sounding becomes unreliable. Under optimum environmental conditions, visual and vibrational orientation have the same relative importance. At the low and the high end of the temperature scale, however, the relative importance of vibrational cues declines significantly.

Temperature dependence of host location is especially relevant when mechanosensory mechanisms are involved because they depend on physical properties of the environment and on the physical activity of the insects (Fischer *et al.*, 2001; Otten *et al.*, 2002; Fischer *et al.*, 2003a). In addition, substantial thermal effects are known for the performance of mechanoreceptors in arthropods (e.g. French, 1985; Coro & Perez, 1990; Franz & Ronacher, 2002). The influence of temperature on mechanosensory host location by vibrational sounding was recently demonstrated in *P. turionellae* as well as in the closely related tropical ichneumonid species *Xanthopimpla stemmator* (Thunberg) (Kroder *et al.*, 2006; Samietz *et al.*, 2006). In both species the percentage of females responding to the vibrational cue and the ovipositor insertion activity, i.e. the average number of ovipositor insertions per responding female, decreased down- and upwards of a

temperature optimum. Also the precision decreases beyond a certain temperature limit – in *P. turionellae* at and below 10°C and at and above 28°C (Samietz *et al.*, 2006). The reliability of receiving vibrations by the mechanoreceptors are presumed to be thermally affected as the wasps improve the quality of self-produced vibrations by an increase of intensity and frequency at low temperatures (Kroder *et al.*, submitted). Although activity of enzymes involved in the visual process may be affected by temperature, for insect vision such negative temperature effects in the studied thermal range are not known (Willmer *et al.*, 2000).

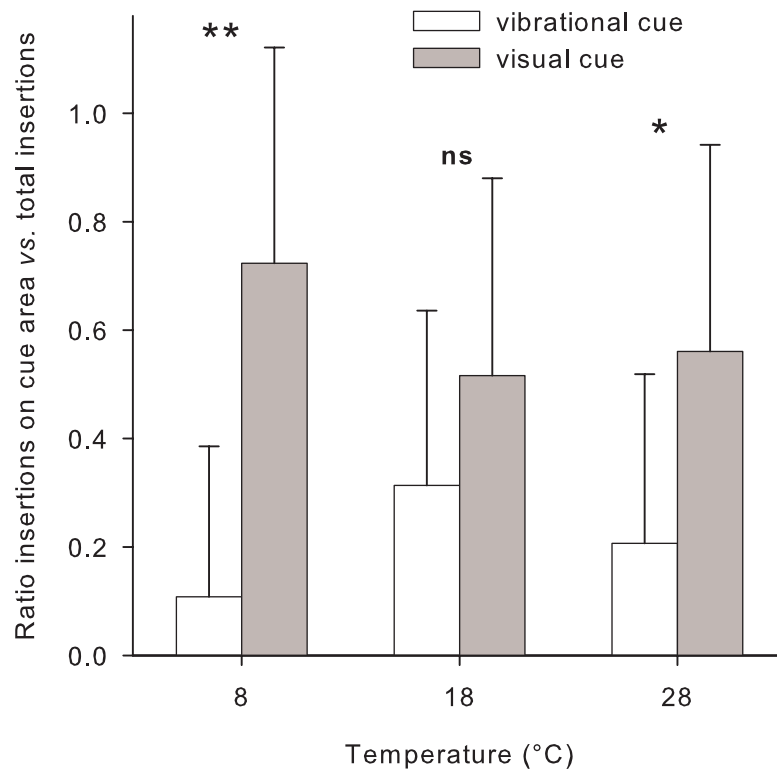


Figure 4. Ratio between number of ovipositor insertions on cue areas and total number of insertions revealing the relative importance of vibrational (white; mean \pm *sd*) and visual cue (grey) in *Pimpla turionellae* at 8, 18 and 28°C. Only females responding with at least three ovipositor insertions were included into this analysis. Asterisks indicate significant differences between vibrational and visual cue by Wilcoxon Signed Ranks test (ns, not significant; *, $P < 0.05$; **, $P < 0.01$).

Few studies have so far investigated the interaction of information from different sensory sources during parasitoid host location on the same spatial scale, and the focus in those studies was on visual combined with chemical cues. The braconid wasp *Microplitis croceipes* shows a stronger preference after conditioning to a combination of visual and chemical cues, than after conditioning to either one alone (Wäckers & Lewis, 1994). In individuals conditioned to both cues, the chemical stimulus was dominant. In the aphidiid wasp *Aphidius ervi*, the importance of visual and chemical perception of the host is displayed in oviposition behaviour at short range (Battaglia *et al.*, 1993; Battaglia *et al.*, 2000). The use of visual and chemical cues in multisensory orientation is further demonstrated in the parasitoid flies *Apocephalus paraponerae* (Morehead & Feener, 2000) and *Exorista mella* (Stireman, 2002). The influence of environmental factors on the multisensory use of information in parasitoid host location was hitherto only investigated with respect to changes of the parasitoids behaviour across the different spatial scales of the host location process (Wellings, 1993; Völkl, 2000; Schwörer & Völkl, 2001).

In multisensory orientation of parasitoids, the role of chemical, visual and vibrational cues varies with the spatial scale (Völkl, 2000; Fischer *et al.*, 2001; Schwörer & Völkl, 2001). Volatile chemicals as well as vision are important for host microhabitat detection (Michaud & Mackauer, 1994; Battaglia *et al.*, 2000; Völkl, 2000) whereas contact chemicals as well as vibrational cues can only be detected after landing on the substrate (Meyhöfer *et al.*, 1997a; Dutton *et al.*, 2000; Fischer *et al.*, 2001; Otten *et al.*, 2002). The females of *P. turionellae* are known to use lightness contrasts in visual host location (Fischer *et al.*, 2004b), and the detection range of achromatic cues is usually larger (Hempel de Ibarra *et al.*, 2001). Consequently, in *P. turionellae* females the orientation towards a potential host habitat – here the plant-stem model – should be primarily visually directed – especially if a visual cue in the habitat is available that may indicate a possible host pupae concealed in the substrate. The wasps then antennate on the plant stem and locate potential hosts by both vibrational sounding and visual orientation. Vision is used both before and after landing on a host patch, whereas mechanosensory information is only accessible after landing. Therefore, the

higher visual responsiveness over all temperatures in our present experiments likely results from the lacking role of vibrational sounding before contact is made with the plant-stem model.

The ovipositor insertion activity of *P. turionellae*, i.e. the average number of insertions per responding female, changes with ambient temperature, even though the parasitoids were allowed to use visual and vibrational cues simultaneously. Such dependence is also revealed in females which exclusively use vibrational sounding during host location (Kroder *et al.*, 2006; Samietz *et al.*, 2006). This analogy between multisensory orientation in the current work and single use of a searching mechanism in previous studies makes evident that insertion activity does not solely depend on a certain sense of host location but also on the general physical activity of the insects. With a higher physical activity at high temperatures the searching process accelerates and allows the wasps to insert the ovipositor more often than at lower temperatures.

In the comparison of the relative importance of cues at medium temperature of 18°C, the *P. turionellae* females showed no preference for one of the cues; they were equally attracted to both, the vibrational and the visual cue. This finding confirms the previously stated interaction between visual orientation and vibrational sounding after landing (Fischer *et al.*, 2001). Decreasing reliability due to changed environmental conditions leads to a decreasing relative importance of that cue. As the reliability of vibrational sounding decreases, the relative importance of visual cues increases at the temperature extremes and especially at the low temperature limit of host location. Consequently non-proportional changes in reliability between two cues used (visual and vibrational) obviously leads the wasps to alter multisensory orientation and switch to the cue, more reliable under given environmental conditions (visual).

Finally, while the precision of vibrational sounding alone is negatively influenced by too low and too high temperatures (Samietz *et al.*, 2006; cf. above), the overall precision of ovipositor insertions in the present study on base of visual and vibrational orientation is not affected by temperature – even when the females of *P. turionellae* switch to only one of the available cues for host location.

These results show that the parasitoids may fully compensate the loss of one cue if another reliable cue is available on the same spatial scale.

9. General Discussion

An influence of environmental temperature on the performance of mechanosensory host location was investigated in ichneumonid parasitoids which attack lepidopteran pupae concealed in plant material. The mechanism of vibrational sounding was tested for thermal effects on reliability in two differently adapted species, and the influence of ambient temperature on vibratory production was studied in one of the two species. Subsequently, possible ways of compensating thermal influences were evaluated and the significance of declining reliability in multisensory orientation was examined. The temperature ranges of successful performance were found to reflect the conditions of natural habitats, and thus, the temperate *Pimpla turionellae* and the tropical *Xanthopimpla stemmator* differed significantly in their temperature ranges of using vibrational sounding. The results of vibration measurements in *P. turionellae* surprisingly revealed an inverse effect of ambient temperature on frequency and intensity, even though body temperature differed only slightly from the environment. Pausing females, by contrast, showed a low-level endothermy as body is warmed up at low ambient temperatures and cooled down at high ambient temperatures. When the parasitoids were allowed to use multiple cues during host location, the relative importance of single cues varied with changing ambient temperature. The role of vibrational sounding decreased in multisensory orientation as its reliability was negatively influenced by temperature.

Temperature range of using vibrational sounding

Host location by vibrational sounding can be affected by ambient temperature in different ways. On the one hand, host location can be quantitatively influenced by activity of the searching wasps. On the other hand, it can be qualitatively influenced with respect to the precision of ovipositor insertions. The parameters quantifying host location activity in this thesis were responsiveness as the proportion of females inserting the ovipositor at least once, and quantitative

insertion activity as the average number of insertions per responding female. The precision was quantified by the bias, i.e. the average deviation of insertions from cue centre, and by the scattering, i.e. the average variance of ovipositor insertions.

In host location activity, the temperate species *P. turionellae* displays an optimum temperature around 18 to 20°C whereas the tropical species *X. stemmator* shows highest responsiveness and insertion activity at 32°C. These optima generally correspond to the thermal conditions of the natural habitats of the two species (Bogenschütz, 1978; Moore & Kfir, 1996). Moreover, responsiveness of vibrational sounding ranges from 6 to 32°C in *P. turionellae* and from 22 to 34°C in *X. stemmator*. Therefore, the results of this thesis verify the parasitoids' adaptation of vibrational sounding to their origins. The temperature in temperate regions can easily vary more than 20°C during the growing season, occasionally during a day, while temperature in tropical regions commonly pivots around 30°C. With respect to the precision, both species are surprisingly able to retain a relatively high precision over a large variation of temperature. In fact, a soundly working host location mechanism is a requirement for successful reproduction to locate the hosts reliable in a wide range of thermal conditions. The insertion precision is kept on the same level and only deteriorates in the temperate species at extremely low and high temperatures. Also, the host location precision reveals the adaptation to temperature of the species since *X. stemmator* is generally more precise than *P. turionellae* at higher temperatures.

Vibrational sounding is suggested to be an analogous adaptation in certain members of the two hymenopteran families Ichneumonidae and Orussidae (Broad & Quicke, 2000). Hence, this sensory system evolved under a variety of climatic conditions and should reflect the temperatures of natural origins. With these findings, it is demonstrated how reliability of this host location mechanism is modified by thermal changes, and in which respect this sensory system is limited.

How to cope with thermal changes

The question arises how the wasps cope with thermal changes to detect the host precisely. One explanation would be an independent body temperature from environmental temperature. By that way, the pupal parasitoids would be able to produce signals in a constant quality and to keep the precision of host location. A temporary endothermy is particularly well studied in bees and bumblebees that achieve body temperatures to approximately 25°C above ambient temperature by shivering wing muscles (Heinrich & Kammer, 1973; Willmer *et al.*, 2000; Stabentheiner, 2001). Although the probability of a strong endothermy is less likely in smaller sized animals (Bishop & Armbruster, 1999), a thermoregulation at a lower level would also facilitate the performance of host location. A varying capability of balancing the body temperature at higher and lower temperatures would, therefore, determine the responsiveness and insertion activity of individuals.

However, measurements on the body temperature of *P. turionellae* during host location by vibrational sounding itself showed no evidence for a thermoregulated body. The searching females have always nearly the same temperature as the surrounding environment irrespective of high or low ambient temperatures. The infrared recordings of pausing females, by contrast, revealed body temperatures that are slightly warmed up at cold ambient temperatures and cooled down at hot ambient temperatures. Vibrational sounding probably conflicts with these endothermic mechanisms as endothermy only occurs in non-searching females. In nature, *P. turionellae* may furthermore regulate its body temperature by behavioural means, which could be combined with low-level endothermy in pausing phases. In behavioural observations, the host-location process was more often discontinued under suboptimal conditions, which could be also caused by thermoregulatory requirements of the wasps.

Despite of changing body temperature, the quality of self-produced vibrations does not drop in *P. turionellae*, neither in time nor in frequency domain. This is actually surprising because signals of other vibratory and auditory systems typically modify the temporal structure, frequency, and

intensity in dependence of body temperature (Shimizu & Barth, 1996; Fonseca & Revez, 2002; Sueur & Sanborn, 2003). Higher temperature elevates the muscle contraction rates and affords the production of higher frequencies with higher intensity (Greenfield, 2002). This temperature dependent variability of intraspecific communication may result in thermal constraints for conspecific recognition. Studies on auditory communication of field crickets report a “temperature-coupling” system in mating behaviour solving these constraints (Pires & Hoy, 1992). As sound rates of the males’ song increase with higher temperature, the females’ phonotactic preference also changes. In *P. turionellae*, an inverse shift of intensity and frequency to temperature is demonstrated in the results of the present study that is hypothetically an adjustment to a negative temperature effect on mechanoreceptors at lower temperatures. Such an adjustment would represent a further kind of temperature-coupling system in which quality of vibrational signals changes as sensitivity of mechanoreceptors diminishes.

Nevertheless, the parasitoids are able to handle the quality of self-produced vibrations without adverse temperature effects. The detailed production of vibrations is still unclear. Own observations and previous studies are not compatible with the following two options (Henaut, 1990; Otten, 2000): (1) percussion of antennae or other body parts against substrate (also known as “drumming”) because one contact of antennae leads to several vibrational pulses, (2) stridulation and tymbal mechanisms because vibrational frequency would be correlated to body size, which is not the case in *P. turionellae*. Consequently, the vibration origin is presumed to be in the insect’s body produced by muscle contractions, and antennal contact is only important for transmission. However, direct myogenic production by continuous muscle contractions is rather unlikely due to the observed independence between temperature and vibrations, and due to the respective frequency in vibrational sounding found in the present study. In this producing option, signal frequency would be expected maximum half as high as the values measured in the present study, like it is demonstrated in many land bugs (Cokl & Virant-Doberlet, 2003). Our results are compatible with a further option for myogenic production of vibrations, i.e. that a single muscle

contraction elicits trains of vibrations (Ewing, 1989). An eigenfrequency could, therefore, be triggered in proper structures of body parts. This hypothesis is in agreement with the slightly warmed body of *P. turionellae* that was recorded during the use of vibrational sounding indicating a low muscle work. The eigenfrequency and the size of these body parts would have to be more or less independent of body size since frequency does not correlate with body size (Otten, 2000). However, at this stage of knowledge the vibrational production in this host location mechanism remains hypothetical and still cannot be answered thoroughly.

Role of vibrational cue in multisensory orientation

In consequence of the low thermal influence on the signal production, the use of vibrational host location is limited by the requirements of mechanoreceptors (cf. above). The temperate species fails to detect potential hosts with a sufficient precision in extreme cold and extreme warm environments. The reliability of vibrational cues declines at these temperatures, and the role of vibrational sounding in a multisensory orientation accordingly decreases. In the final experiments of the present thesis, *P. turionellae* was allowed to employ vibrational and visual orientation at short-range host location. Under suboptimal conditions, wasps switched from the use of multiple cues to the single use of the more reliable visual cue. This matches our expectations that the relative importance of cues varies at the same spatial scale depending on their reliability. Such a phenomenon can be also assumed at higher spatial scales of host location as efficiency of involved sensory systems largely succumbs to environmental factors. For example, visual orientation certainly depends on illumination intensity, propagation of volatile chemicals is influenced by wind, and contact chemicals can be washed away by rain water. The varying relative importance has so far been focused on the dependence on the range of host location (Battaglia *et al.*, 2000; Morehead & Feener, 2000; Völkl, 2000). Based on our results, relative importance of cues does not only vary in spatial scales, and this actually constitutes a further aspect of the reliability–detectability syndrome in host

location (Vet *et al.*, 1991). The simultaneous use of several information sources not only improves the reliability, it also enables parasitoids to keep accuracy and efficiency of host location despite of changing abiotic factors. Hence, due to multisensory orientation, searching behaviour is not restricted to possibly narrow windows of environmental conditions for the use of single information sources.

Finally, the described host location strategy is highly efficient to detect concealed host pupae in a microhabitat. The pupal parasitoids using this strategy seem to deal easily with challenges of reduced cue availability and adverse weather conditions. With respect to the other side of the parasitoid-host relationship, there seems to be no way out for the pupae if parasitoid searches at the host's microhabitat. Yet, the efficiency of vibrational sounding strongly depends on substrate density (Fischer *et al.*, 2003a), and an increasing mechanical resistance by being deep in a substrate, is a possible response to this mechanism. Besides, less is known about the host habitat location of these parasitoids on larger spatial scale, i.e. long-range host detection. Both species, *P. turionellae* and *X. stemmator*, attack plenty of lepidopteran pupae and should not be specialized on host-specific cues in this stage of the searching process. Such polyphagous parasitoids are supposed either to use more general and less reliable cues or to find randomly host-containing patches (Vet & Dicke, 1992). Considering the entire foraging procedure from host habitat selection to host acceptance and parasitization, especially the constant functioning of short-range mechanisms are presumably substantial for these parasitoids.

10. References

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12. Curriculum vitae

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