

Listening when there is no sexual signalling? Maintenance of hearing in the asexual bushcricket *Poecilimon intermedius*

Gerlind U. C. Lehmann · Johannes Strauß ·
Reinhard Lakes-Harlan

Received: 25 October 2006 / Revised: 12 December 2006 / Accepted: 7 January 2007
© Springer-Verlag 2007

Abstract Unisexual reproduction is a widespread phenomenon in invertebrates and lower vertebrates. If a former sexual reproducing species becomes parthenogenetic, we expect traits that were subject to sexual selection to diminish. The bushcricket *Poecilimon intermedius* is one of the few insect species with obligate but diploid parthenogenetic reproduction. We contrasted characters that are involved in mating in a sexually sibling species with the identical structures in the parthenogenetic *P. intermedius*. Central for sexual communication are male songs, while receptive females approach the males phonotactically. Compared to its sister-species *P. ampliatus*, the morphology of the hearing organs (acoustic spiracle, crista acustica) and the function of hearing (acoustic threshold) are reduced in *P. intermedius*. Nonetheless, hearing is clearly maintained in the parthenogenetic females. Natural selection by acoustic hunting bats, pleiotropy or a developmental trap may explain the well maintained hearing function.

Keywords Neuroanatomy · Bioacoustics · Auditory ecology · Regression · Vestigialization

G. U. C. Lehmann (✉)
Institut für Zoologie, AG Evolutionsbiologie,
Freie Universität Berlin,
Abteilung Evolutionsbiologie,
Königin-Luise-Straße 1-3,
14195 Berlin, Germany
e-mail: gerlind.lehmann@t-online.de

J. Strauß · R. Lakes-Harlan
Institut für Tierphysiologie,
AG Integrative Sinnesphysiologie,
Universität Gießen, Gießen, Germany

Introduction

Traits under relaxed selection are expected to become reduced or disappear completely, a process called vestigialization. Vestigialization is a widespread phenomenon that highlights the importance of natural selection for the maintenance of adaptive traits (Fong et al. 1995; Porter and Crandall 2003). Vestigialization could proceed more quickly if trait reduction was associated with increased fitness. Reduction of the vestigial trait may be selected directly if the trait becomes a fitness liability, like some sexually selected traits (Wiens 2001). Loss of traits has been reported in a wide variety of organisms, such as cave-dwelling animals (Wilkins et al. 2000), subterranean mammals (Nevo 1999) or island birds in the absence of predators (McNab 1994).

In parthenogenetic species or populations, traits historically involved in sexual reproduction are no longer under selection and potentially subject to vestigialization (Carson et al. 1982). Asexuality may directly affect the mutation rate due to the loss of the sexual phase and lead to the cessation of sexual selection (Normark et al. 2003).

The evolution of signalling is at the heart of many evolutionary discussions (Greenfield 2002). Hearing in Orthoptera is associated with two functions: intraspecific communication (e.g. mate recognition and localization) and predator recognition and avoidance (Bailey 1991; Gwynne 2001; Gerhardt and Huber 2002; Robinson and Hall 2002). Both functions might be driven by different requirements on auditory performance and can be caused by distinct processes—sexual selection (in the case of intraspecific communication) and more general natural selection. It is argued that in a variety of cases the actual structure and performance of the

acoustic system reflects merely the balance between sexual selection due to elaborated signalling and a counteracting natural selection (Zuk and Kolluru 1998; Greenfield 2002). Thus, their respective influence on a given hearing system is hard to distinguish causally.

The tympanal auditory system of tettigoniid grasshoppers is a highly ordered sensory system (Römer 1983; Lakes and Schikorski 1990). The receptors are located in a linear array of chordotonal cells, called the *Crista acustica*. The airborne sound is transferred to the receptors by a highly specialized auditory spiracle or trachea (Nocke 1975; Hoffmann and Jatho 1995). Tibial tympana contribute to the sensitivity of the system (Bangert et al. 1998).

Evidence exists that intraspecific communication in tettigoniids evolved before predation by ultrasound emitting bats (Stumpner and von Helversen 2001), but the influence of predation on audition is not easily quantified (Hoy 1992). Since the Miocene bats have become a significant predation risk and can be considered as a major force in shaping acoustic behaviour in tettigoniids (Hoy 1992; Yager 1999; Lehmann 2003; Fullard et al. 2004).

The ensiferan genus *Poecilimon*, including more than 120 species, has served as model for evolutionary studies (Heller 1997). The bushcricket *P. intermedius* (Fieber, 1853) is a good system to investigate the influence of sexual and natural selection on the hearing system. In *P. intermedius* only females are known over its distribution range (Heller and Lehmann 2004) and these females are diploids (Warchalowska-Sliwa et al. 1996). In rearing experiments, this species was capable of all female reproduction and could not be treated to revert to sexual reproduction (Lehmann and Bourtzis, submitted). Parthenogenesis in *P. intermedius* can be classified according to Suomalainen et al. (1987) as constant obligate thelytoky (unfertilised eggs develop into females) and somatic diploidy. The ecological pattern is geographical parthenogenesis (Peck et al. 1998; Kearney 2005), with a more northerly and larger distribution area than sexual *Poecilimon* species (Heller and Lehmann 2004).

As *P. intermedius* is an obligate parthenogenetic, the intraspecific role of acoustic communication in mate recognition and finding is lost and therefore sexual selection pressure on auditory performance can be assumed to no longer exist. Reduction could act on several levels of the hearing system, from the sensory structures to the neuronal network in the central nervous system (CNS). The objectives of the present study were the analysis of peripheral sensory structures, which provide input for higher integration centres. Comparing the parthenogenetic species *P. intermedius*

with its comparatively recently separated closest relative *P. ampliatus*, identified by DNA sequencing (Lehmann et al., submitted), allows the comparison of auditory system structure and function in presence and absence of intraspecific communication. For the first time two sister-species with alternative reproductive modes were tested for the design of the hearing structures as the acoustic spiracle, the entrance of sound into the trachea, and the tympana as well as the neuroanatomy of the *crista acustica*. Electrophysiological recordings of auditory thresholds determined physiological performance. We expect the auditory performance of *P. intermedius* to be reduced in sensitivity compared to its sister-species *P. ampliatus*.

Materials and methods

The members of the *P. ampliatus*-group are medium size, flightless bushcrickets (Heller and Lehmann 2004). Adult *P. intermedius* (FIEBER, 1853) and *P. ampliatus* BRUNNER VON WATTENWYL, 1878 were individually housed in 200 ml boxes (Drosophila rearing boxes, Greiner Bio-one GmbH, Frickenhausen, Germany; <http://www.GreinerBioone.com>) and provided with a mixture of *Taraxacum* leaves and flowers and mixed species pollen available from a health food store ad libitum.

Morphology

The size of the acoustic spiracle was determined using scanning electron microscopy. Females of both species were decapitated and the ventral lobe of the pronotum was removed to expose the spiracle. The isolated thoraces with the visible spiracle were transferred to small caps covered with 10- μ m gauze and dehydrated in increasing concentrations of ethanol from 70 to 100%. Critical point drying was performed with CO₂ (Balzers CPD 030, Balzers AG, Lichtenstein). The specimen were fixed on stubs with double-sided adhesive tape and coated with gold (Balzers SPD 040). Pictures were obtained by a Philips SEM 515. Digitalized pictures of the spiracles were measured, using the program Scion Image Beta 4.03 for Windows (<http://www.scion-corp.com>). As references for body size we measured the length of the hind femur and the pronotum for all females with a Mitutoyo calliper rule (accuracy 0.01 mm).

The size the tympana were measured with help of a microscope (Leitz), using an ocular micrometer (accuracy 0.025 mm). The maximum extensions in the proximo-distal and the dorso-ventral axis were measured

for both anterior and posterior tympana, respectively. Some tympana were processed for scanning electron microscopy as described above.

Neuroanatomy

For marking the sensory cells of the complex tibial organ, tympanal nerves were backfilled with a CoCl_2 solution (Lakes-Harlan et al. 1991). Forelegs were cut off and attached with needles to a silicone covered petri dish bottom. The tympanal nerve was dissected from the femur and placed into a glass capillary filled with 5% CoCl_2 and incubated at 4°C for 2 days. After incubation, the tarsus was cut off and the tibia was opened dorsally. The CoCl_2 was precipitated with ammonium sulphide (1% solution for 10 min), and the preparations were fixed in 4% paraformaldehyde. The neuronal marking was enhanced by the silver intensification method (Bacon and Altman 1977). All preparations were dehydrated (in 30, 50, 70, 90, 96, 100% Ethanol for at least 60 min each) and cleared in methyl salicylate. The complex tibial organ was documented by pictures and drawings using a Leitz Dialux microscope and a drawing tube ($n = 3$ for *P. ampliatus* and $n = 5$ for *P. intermedius*).

Hearing threshold

For electrophysiological recordings, animals were fixed on their back on a metal holder (seven females of each species). The forelegs were attached with wax to metal wires, resembling the normal leg position. The recording took place in a Faraday cage that was covered with sound dampening material.

Stimulation Acoustic stimuli were delivered from a broad-band loudspeaker (Dynaudio, DF 21/2) located at a distance of 38 cm from the animal. The stimuli were computer generated and amplified (Lang et al. 1993). Frequencies between 3 and 40 kHz were presented to the animals. Each frequency–intensity combination was tested 5 times with a stimulus duration of 100 ms each. The intensity increased from 30 to 50 dB SPL in steps of 5 dB and from 50 to 80 dB in steps of 10 dB SPL. Calibration measurements were carried out with a sound level meter (Brüel and Kjær 2203) and a microphone (Brüel and Kjær 4135).

Recordings Summed action potentials from the tympanal nerve were recorded near the entrance of the nerve into the prothoracic ganglion. Recordings were carried out with a silver wire hook electrode. The reference electrode was inserted between the connectives close to the recording site. The signal was amplified 1,000 fold by a preamplifier (Tektronix T122) and displayed on an oscil-

loscope and transferred to earphones. The auditory threshold was determined acoustically and visually as the first signal intensity, which excited a neuronal response.

Results

Spiracle size

The general size of *P. intermedius* females was smaller than in *P. ampliatus* females, measured as pronotum (4.65 ± 0.17 mm vs. 5.33 ± 0.25 , T test: $T = 8.73$, $df = 28$, $P < 0.001$) and hind femur length (14.98 ± 0.48 mm vs. 16.20 ± 0.44 , T test: $T = 7.23$, $df = 28$, $P < 0.001$). Despite this size difference between the species, the correlation between hind femur and spiracle size is only weak (Fig. 1, correlation coefficient for both species combined: $R^2 = 0.18$) and even less when analysed separately for either *P. intermedius* ($R^2 = 0.06$) or *P. ampliatus* ($R^2 = 0.09$).

The general auditory anatomy of females of both *P. intermedius* and *P. ampliatus* is typical of tettigoniid bushcrickets. The tracheal system of the prothoracic leg is expanded in the tibia to form air-filled vesicles, which lie beneath the two tympanal membranes. The leg trachea opens to the exterior through an acoustic spiracle. This acoustic spiracle on the lateral side of the prothorax functions as input for the hearing system (Fig. 2). The acoustic spiracle has a comparatively small opening in *P. intermedius* of mean (\pm SD) 0.94 ± 0.17 mm² ($n = 17$). As such the spiracle is significantly smaller than in the sister-species *P. ampliatus* (1.11 ± 0.16 mm², $n = 13$) (T test: $T = 2.70$, $df = 28$, $P < 0.05$). Females of *P. intermedius* have a 15% smaller spiracle size in comparison to *P. ampliatus*

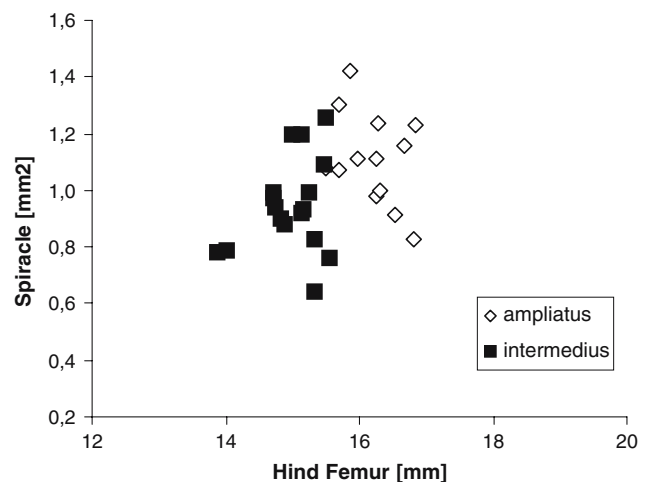


Fig. 1 Spiracle size compared to length of the hind femur in females of *P. intermedius* ($n = 17$) and *P. ampliatus* ($n = 13$)

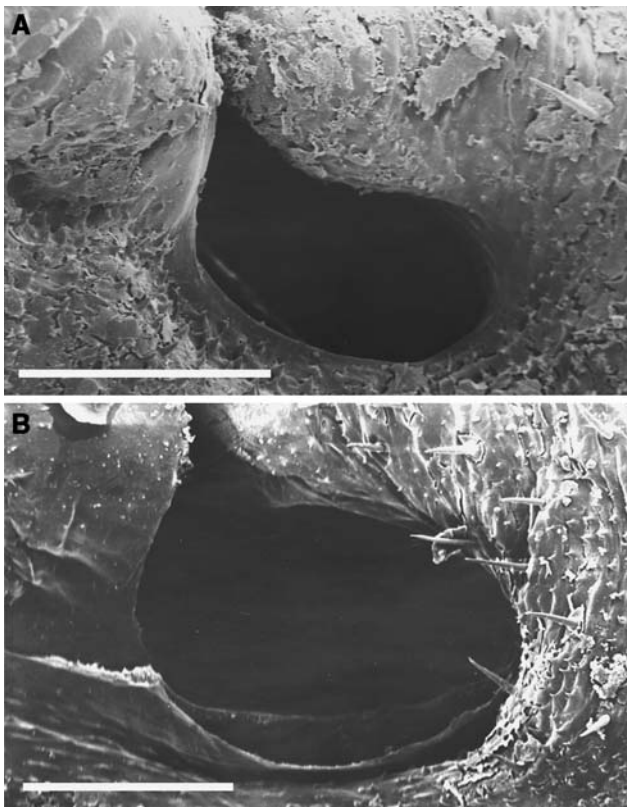


Fig. 2 SEM picture of the acoustic spiracle of a female of *P. intermedius* (a) and *P. ampliatus* (b). Scale bar 0.1 mm

females (Fig. 3). Nonetheless, the variance within individuals was similar for both species (Bartlett test for equal variances: $\chi^2 = 0.14$, $df = 1$, $P = 0.71$ NS).

Tympanum size

The most obvious structures of the auditory system in the forelegs of bushcrickets are two tympanic

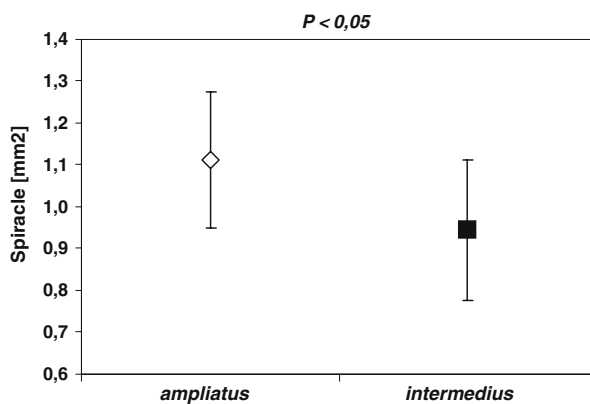


Fig. 3 Area of the acoustic spiracle of the *P. ampliatus* ($n = 13$) and *P. intermedius* ($n = 17$), given as means (\pm SD)

membranes on the anterior and posterior side beneath the knee (Fig. 4). The size of the tympana was identical between the two species (see Table 1) for the proximo-distal axis (two-way ANOVA: species: $F_{1,32} = 1.81$, $P = 0.18$ NS) and the dorso-ventral axis ($F_{1,32} = 0.00$, $P = 0.96$ NS) for both, the anterior and the posterior membrane. However, the anterior tympana were significantly larger than the posterior membranes in both species in the proximo-distal axis ($F_{1,32} = 42.12$, $P < 0.001$), as well as in the dorso-ventral axis ($F_{1,32} = 14.08$, $P < 0.001$). The interaction term was not significant for both measurement axes, which indicates that there was no influence of the species on the size reduction of the posterior tympana compared to the anterior side (proximo-distal axis: $F_{1,32} = 0.84$, $P = 0.36$ NS, dorso-ventral axis: $F_{1,32} = 2.97$, $P = 0.09$ NS).

Neuroanatomy of the Crista Acustica

The general organization of the three receptor organs within the proximal tibia of both *P. ampliatus* and *P. intermedius* is the same as in most other tettigoniids. The complex tibial organ is divided into three sensory organs: the subgenual organ, the intermediate organ and the crista acustica (Fig. 5). The subgenual organ forms a “sail”-like structure extending from anterior into the haemolymph channel of the leg. In both species, the



Fig. 4 Tympanum of *P. intermedius* female anterior. The membrane is located just under the knee, which is to the top. Scale bar 0.1 mm

Table 1 Tympana sizes given as means [\pm SD] in mm for the two species

	<i>n</i>	Anterior tympanum		Posterior tympanum	
		Proximo-distal	Dorso-ventral	Proximo-distal	Dorso-ventral
<i>P. intermedius</i>	11	0.75 \pm 0.02	0.31 \pm 0.02	0.63 \pm 0.04	0.28 \pm 0.02
<i>P. ampliatus</i>	23	0.75 \pm 0.05	0.30 \pm 0.02	0.67 \pm 0.09	0.29 \pm 0.02

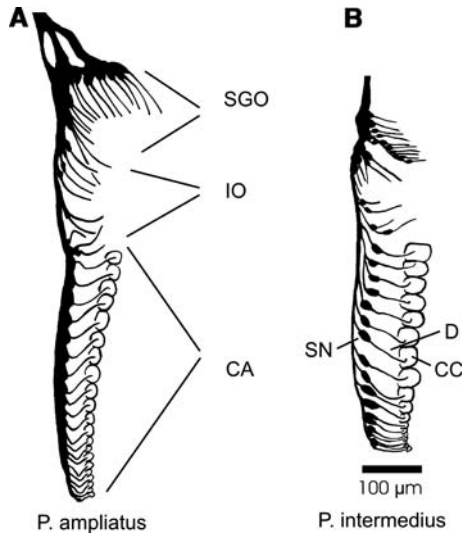


Fig. 5 Drawings of the array of the receptor cells of the complex tibial organ in the foreleg of a female of *P. ampliatus* (a) and *P. intermedius* (b). The three parts of the organ are marked for *P. ampliatus* (CA crista acustica, IO intermediary organ, SGO subgenual organ). A sensory neuron (SN), with its dendrite (D) and a cap cell (CC) of a scolopidium is indicated exemplarily in the crista acustica of *P. intermedius*

anterior scolopidial receptor cells stretch along the sail and probably react to vibrational stimuli. The intermediate organ is similar in both species and contains about 10–11 scolopidial receptor cells. The crista acustica (Fig. 6) is undoubtedly the most interesting part of the sense organ array as its primary function in tettigoniids is to transduce airborne sound. The receptor cells form a linear row along the length axis of the leg. Generally, the dendrites of the sensory cells as well as their cell bodies become smaller towards the distal end of the crista acustica. The decrease of size is even more marked in the cap cells to which the sensory neurons are attached (Fig. 6). Usually in both species about six to eight cap cells at the distal end are much smaller than the proximal cells. Females of *P. intermedius* have a lower number of receptor cells in the crista acustica (16–18 cells, *n* = 5) in comparison to those of *P. ampliatus* (21 cells, *n* = 3) (Fig. 5). The overall length of the crista acustica is in mean (\pm SD) 0.45 \pm 0.04 mm in *P. ampliatus* (*n* = 3) and 0.35 \pm 0.03 mm in *P. intermedius* (*n* = 2). Even with such a small sample size this difference is significant (*T* test: *T* = 3.43, *df* = 3, *P* < 0.05).

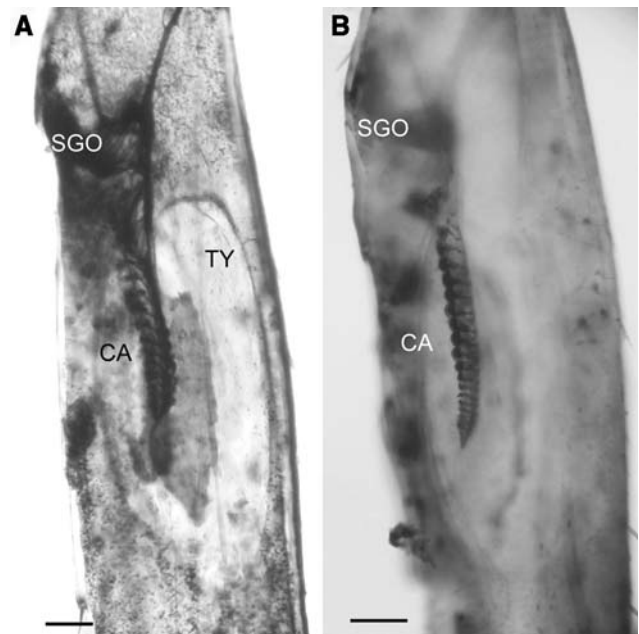


Fig. 6 Lateral view of complex tibial organ and the crista acustica in a foreleg of *P. intermedius* (left) and *P. ampliatus* (right), labeled with cobalt chloride. Scale bar 0.1 mm

Hearing threshold

Extra cellular recording the tympanal nerve show that both species perceive auditory stimuli. *P. ampliatus* has a low acoustic threshold of, meaning that the nerve is most sensitive, between 7 and 10 kHz (Fig. 7). The energy of the male song is mainly in the range between 20 and 40 kHz (Heller 1988). In this range the threshold for nervous response is about 55–60 dB SPL, indicating a mismatch in tuning to the male song spectrum. *P. intermedius* females have a hearing threshold with some small differences to that of *P. ampliatus* (Fig. 7). Auditory tuning is broad, and the threshold curve is rather flat, lacking a clear sensitivity peak. Most sensitive hearing is around 10 kHz and around 65 dB SPL at high frequencies used in sexual species to attract mates. On average, the hearing threshold in *P. intermedius* females was 3–7 dB higher than in *P. ampliatus* females at all frequencies, with significant differences in all frequency ranges. This difference corresponds well with the reduced spiracle size and reduced number of sensory cells in *P. intermedius*. However, both *P. ampliatus* and the parthenogenetic species

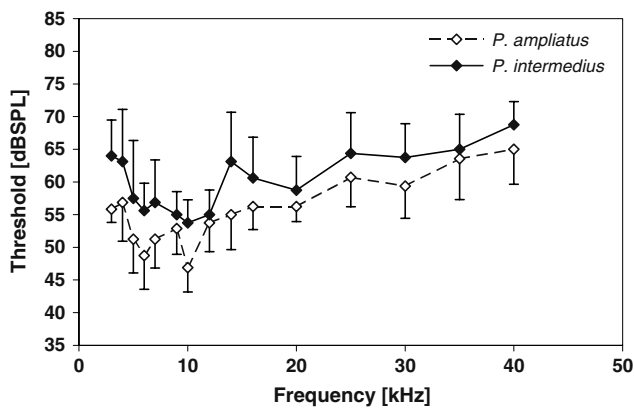


Fig. 7 Mean auditory threshold curves (\pm SD) for *P. ampliatus* ($n = 8$) and *P. intermedius* ($n = 8$)

P. intermedius showed reduced sensitivity, especially at higher frequencies, compared to bushcrickets with larger spiracles (Stumpner and Heller 1992).

Discussion

As *P. intermedius* is obligatory parthenogenetic, the intraspecific role of acoustic communication in mate recognition and finding (Gwynne 2001) is lost, and therefore sexual selection pressure on auditory performance no longer exists. Natural selection due to predator recognition may still be acting on the tympanal organ. As expected, females of the parthenogenetic *P. intermedius* show reduction in the hearing structures compared to females of its sexual sister-species *P. ampliatus*. However, the anatomical and functional vestigialization is rather small, given the complete loss of intraspecific acoustic communication. The split of the two species is of comparatively young age, given the low genetic differences (Lehmann et al., MS). The inferred age of separation of the split between the two species might be assumed up to 200,000 years, leaving enough time for a significant respond to the altered reproductive mode. However, the origin of parthenogenesis in *P. intermedius* might be younger than the split.

In both species the complex tibial organ characteristic for Tettigoniid bushcrickets (Lakes and Schikorski 1990) is fully developed. Tympana are present and do not show size differences between the sister-species. The total size of the tympana in both these species and other *Poecilimon* species (Stumpner and Heller 1992) is small compared to other bushcrickets (Bangert et al. 1998). In both *P. intermedius* and *P. ampliatus* the crista acustica has a relatively small number of receptor cells compared to Tettigoniids in general, which often possess 20–40 cells (Lakes and Schikorski 1990; Robin-

son and Hall 2002). The oak bushcricket *Meconema thalassinum* (Schumacher, 1973, 1979), which has changed intraspecific communication towards leaf drumming (Sismondo 1980), and the Australian *Phasmodes ranatiformes* (Lakes-Harlan et al. 1991), which is an atympanate species, have both receptor cell numbers as low as the asexual *P. intermedius*. *P. intermedius* has on average four receptor cells less than its sister-species *P. ampliatus*. Such a difference is rather large, given that bushcricket species in a genus differ in mean by two (*Ephippiger*: Schumacher 1973, 1979) or just one (*Tettigonia*: Kalmring et al. 1995) receptor cell in the crista acustica. This reduction in cell number is a clear evidence of vestigialization (Fong et al. 1995).

Variation of a vestigialized trait might be greater as an effect of a gradual reduction process, leaving traces of the reduction steps behind (West-Eberhardt 2003). In contrast parthenogenetic reproduction is said to be connected with lower variation, due to the lack of recombination (Felsenstein 1974). The intraspecific variation of the number of crista receptors is usually restricted to 1 or 2 cells (Lakes and Schikorski 1990; Robinson and Hall 2002), to which the data from both *P. ampliatus* and *P. intermedius* accord. Therefore the degree of variation seems unaffected by the strong vestigialization process of the cell number.

The acoustic spiracle is the main source of sound entry in bushcrickets (Robinson and Hall 2002). These spiracles are smaller in the two species we studied than in any known bushcrickets of similar size (Bangert et al. 1998). Only the Australian *Kawanaphila* species have similar small openings (Bailey and Römer 1991; Mason and Bailey 1998), but they are much smaller, weighing only 10–20% of our species (Simmons and Bailey 1990; Lehmann and Lehmann 2007). The differences between *P. ampliatus* and *P. intermedius* in spiracle size are, even if significant, rather small. Nonetheless, this small difference correlates with a reduced auditory performance of *P. intermedius*.

Both *Poecilimon* species hear airborne sound over the entire spectrum tested, showing relatively high hearing thresholds, meaning less sensitivity, compared to other Tettigoniids, which can achieve thresholds at intensities of 25–30 dB SPL (Gerhardt and Huber 2002). This overall low sensitivity fits to the relatively small spiracle, as hearing threshold and spiracle size were closely linked in three other *Poecilimon* species (Stumpner and Heller 1992).

Evolutionary implications of reduction

Natural selection tends to act most strongly on aspects of the phenotype at relatively high levels of biological

organization because they are the most strongly correlated with fitness (Ketterson and Nolan 1999; Irschick and Garland 2001; Kingsolver and Huey 2003; Costa and Sinervo 2004). Complex traits with many subordinate traits at lower levels of biological organization may be affected by natural selection (Swallow and Garland 2005). Thus, the evolutionary response to selection on such complex traits necessarily entails associated changes in aspects of morphology, physiology and biochemical pathways (Ghalambor et al. 2003; Sinervo and Calsbeck 2003).

Reduction in the functionality of a trait can affect afferent sensory structures, central neural structures, and effectors like muscles or glands (Katz and Harris-Warwick 1999). For tympanal organs, reduction has occurred in several insect taxa (Stumpner and von Helversen 2001). For those investigated in detail with respect to neuroanatomy and physiology, different levels of reduction have been documented, which ultimately affect the species specific behaviour. One is the loss of behavioural responses to auditory stimuli that have lost their biological significance, as observed in a moth (Fullard et al. 2004). Females of *P. intermedius* did not show phonotaxis when presented with the *P. ampliatus* male song (Lehmann MS). In species of the *P. propinquus*-group, female song preferences were not species-specific, but correspond well with the similarity of song patterns produced by conspecific and heterospecific males (A.W. Lehmann, personal communication, 2006). Additionally, peripheral structures can be reduced even to the obliteration of tympana and reduction of auditory trachea as in the mimetic tettigoniid *Phasmodes* (Lakes-Harlan et al. 1991). Comparative studies have led to the conclusion that such structures can be readily modified by evolution, while neuronal sensory structures are more conservative and cannot (Dumont and Robertson 1986; Yager 1999). Even in a secondarily atympanate species of Tettigoniidae the crista acustica is present (Lakes-Harlan et al. 1991), which indicates evolutionary conservatism in this structure.

The basic organization of the nervous systems tends to be highly conservative (Katz and Harris-Warwick 1999). In invertebrates, where individual neurons can be unambiguously identified from animal to animal within a species, homologous neurons can be identified in disparate groups of insects (Edwards 1997). These neurons can exhibit certain morphological modifications that are taxon specific (Buschbeck and Strausfeld 1996). The most common difference seen in closely related species is a change in the number of cells of a particular type. The organizational features of some neuronal structures seems to provide a simple mecha-

nism for addition or subtraction of identical units, leading to computationally important changes in neuronal number (review in Katz and Harris-Warwick 1999).

Plasticity in *P. intermedius* follows a pattern of vestigialization of the accessory spiracle and receptor number that maintains hearing but affects sensitivity. The reduced hearing threshold does not imply changed central organization but follows from a reduced input by the smaller spiracle. It will be interesting to evaluate possible changes in the central neuronal network of auditory processing. The basic auditory networks are known in tettigoniids (Stumpner 1996) and changes might be followed to a single cell level. In other insects like moths (Fullard et al. 2004) behavioural changes in a predator free islands have been documented, with the loss of a defensive behaviour against bat ultrasound emissions. While the single auditory receptor neuron still exists and possesses similar sensitivity thresholds compared to the cell of recently introduced species, it exhibits reduced firing activity (Fullard et al. 2007). Similar to our bushcricket species, in this moth system the neural regression is incomplete and the sensori-behavioural integration decays gradually following the removal of the selection pressure exerted by echolocating bats.

Two evolutionary processes can underlie the reduction in sensory structures such as tympanal organs. These are adaptive and random changes (Fong et al. 1995; Porter and Crandall 2003). Adaptation would reduce sensory organs/structures in order to save energy and material by not building and maintaining them. Random mutations could equally lead to a reduced organ once selection pressure is weakened after a reduced biological function. For the *Poecilimon* species reported here, the causation of reduction is not easily identified. *P. intermedius* has four crista receptors less than *P. ampliatus*, which is a 19% reduction, but still covers the entire frequency range tested. The reduced sensitivity correlates with the spiracle size. As the crista may still function in predation avoidance, maintenance of the hearing organ would still be caused by natural selection. Ultrasound emitting bats are regarded as a strong natural selection force in maintenance of hearing function in insects (Hoy 1992; Yager 1999). In bushcrickets acoustic perception serves both anti-predatory and sexual functions. If anti-predator functions are relatively strong, the evolutionary loss of sexual signalling by parthenogenesis may not be accompanied by a major reduction in hearing as found in *P. intermedius*. How strong this selection pressure actually is has to be demonstrated experimentally, but following our results we assume natural selection to be a stronger force than sexual selection.

Acknowledgments We thank K.-G. Heller, A.W. Lehmann and R. Hickson for their discussion of the manuscript. GL is supported by the “Berliner Programm zur Förderung der Chancengleichheit für Frauen in Forschung und Lehre” of the Humboldt University Berlin. The experiments comply with the principles of animal care and also with current laws of the Federal Republic of Germany.

Reference

- Bacon JP, Altman JS (1977) A silver intensification method for cobalt-filled neurons in wholemount preparations. *Brain Res* 138:359–363
- Bailey WJ (1991) Acoustic behaviour of insects. Chapman and Hall, London
- Bailey WJ, Römer H (1991) Sexual differences in auditory sensitivity: mismatch of hearing threshold and call frequency in a tettigoniid (Orthoptera, Tettigoniidae: Zaprochilinae). *J Comp Physiol A* 169:349–353
- Bangert M, Kalmring K, Sickmann T, Stephen R, Jatho M, Lakes-Harlan R (1998) Stimulus transmission in the auditory receptor organs of the foreleg of bushcrickets (Tettigoniidae) I. The role of the tympana. *Hearing Res* 115:27–38
- Buschbeck EK, Strausfeld NJ (1996) Visual motion-detection circuits in flies: small-field retinotopic elements responding to motion are evolutionarily conserved across taxa. *J Neurosci* 16:4563–4578
- Carson HL, Chang LS, Lyttle TW (1982) Decay of female sexual behaviour under parthenogenesis. *Science* 218:68–70
- Costa DP, Sinervo B (2004) Field physiology: physiological insights from animals in nature. *Ann Rev Physiol* 66:209–238
- Dumont JPC, Robertson RM (1986) Neuronal circuits: an evolutionary perspective. *Science* 233:849–853
- Edwards JS (1997) The evolution of insect flight: implications for the evolution of the nervous system. *Brain Behav Evol* 50:8–12
- Felsenstein J (1974) The evolutionary advantage of recombination. *Genetics* 78:737–756
- Fong DW, Kane TC, Culver DC (1995) Vestigialization and loss of nonfunctional characters. *Ann Rev Ecol Syst* 26:249–268
- Fullard JH, Ratcliffe JM, Soutar AR (2004) Extinction of the acoustic startle response in moths endemic to a bat-free habitat. *J Evol Biol* 17:856–861
- Fullard JH, Ratcliffe JM, ter Hofstede HM (2007) Neural evolution in the bat-free habitat of Tahiti: partial regression in an antipredator auditory system. *Biol Lett* 3:26–28
- Gerhardt HC, Huber F (2002) Acoustic communication in insects and anurans—common problems and diverse solutions. University of Chicago Press, Chicago
- Ghalambor CK, Walker JA, Reznick DN (2003) Multi-trait selection, adaptation, and constraints on the evolution of burst swimming performance. *Integr Comp Biol* 43:431–438
- Greenfield MD (2002) Signalers and receivers. Oxford University Press, Oxford
- Gwynne DT (2001) Katydid and bush-crickets. Reproductive behaviour and evolution of the Tettigoniidae. Comstock, Ithaca
- Heller K-G (1988) Zur Bioakustik der Europäischen Laubheuschrecken. Josef Margraf, Weikersheim
- Heller K-G (1997) Geld oder Leben - die unterschiedlichen Kosten des Gesangs bei Laubheuschrecken. *Jb Akad Wiss Göttingen* 1997:132–152
- Heller K-G, Lehmann A (2004) Taxonomic revision of the European species of the *Poecilimon ampliatus*-group (Orthoptera Phaneropteridae). *Mem Soc Entomol Ital* 82:403–422
- Hoffmann E, Jatho M (1995) The acoustic trachea of Tettigoniids as an exponential horn: theoretical calculations and bioacoustical measurements. *J Acoust Soc Am* 98:1845–1851
- Hoy RR (1992) The evolution of hearing in insects as adaptation to predation from bats. In: Webster DB, Fay RR, AN Popper (eds) The evolutionary biology of hearing. Springer, Heidelberg, pp 115–129
- Irschick DJ, Garland T (2001) Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Ann Rev Evol Syst* 32:367–396
- Kalmring K, Rössler W, Jatho M, Hoffmann E (1995) Comparison of song frequency and receptor tuning in two closely related bushcricket species. *Acta Biol Hung* 46:457–469
- Katz PS, Harris-Warwick RM (1999) The evolution of neuronal circuits underlying species-specific behaviour. *Curr Opin Neurobiol* 9:628–633
- Kearney M (2005) Hybridization, glaciation and geographic parthenogenesis. *Trends Ecol Evol* 20:495–502
- Ketterson ED, Nolan V (1999) Adaptation, exaptation, and constraint: a hormonal perspective. *Am Nat* 154:S4–S25
- Kingsolver JG, Huey RB (2003) Introduction: the evolution of morphology, performance, and fitness. *Integr Comp Biol* 43:361–366
- Lakes R, Schikorski T (1990) Neuroanatomy of Tettigoniids. In: Bailey WJ, Rentz DCF (eds) The Tettigoniidae: biology, systematics and evolution. Crawford House, Bathurst, pp 166–190
- Lakes-Harlan R, Bailey WJ, Schikorski T (1991) The auditory system of an atympanate bushcricket *Phasmodes ranatiformes* (Westwood) (Tettigoniidae: Orthoptera). *J Exp Biol* 158:307–324
- Lang F, Brandt G, Glahe M (1993) A versatile multichannel stimulator controlled by a personal computer. In: Elsner N, Heisenberg M (eds) Gene, brain, behaviour. Thieme Verlag, Stuttgart, p 892
- Lehmann GUC (2003) Review of biogeography, host range and evolution of acoustic hunting in Ormiini (Insecta, Diptera, Tachinidae), parasitoids of night-calling bushcrickets and crickets (Insecta, Orthoptera, Ensifera). *Zool Anz* 242:107–120
- Lehmann GUC, Lehmann AW (2007) Sex differences in “time out” from reproductive activity and sexual selection in male bushcrickets (Orthoptera: Zaprochilinae: *Kawanaphila mirila*). *J Insect Behav* (in press)
- Mason A, Bailey WJ (1998) Ultrasound hearing and male–male communication in Australian katydids (Tettigoniidae: Zaprochilinae) with sexually dimorphic ears. *Phys Entomol* 23:139–149
- McNab KB (1994) Energy conservation and the evolution of flightlessness in birds. *Am Nat* 144:628–642
- Nevo E (1999) Mosaic evolution of subterranean mammals: regression, progression, and global convergence. Oxford University Press, Oxford
- Nocke H (1975) Physical and physiological properties of the tettigoniid (“grasshopper”) ear. *J Comp Physiol A* 100:25–57
- Normark BB, Judson OP, Moran NA (2003) Genomic signatures of ancient asexual lineages. *Biol J Linn Soc* 79:69–84
- Peck JR, Yearsley JM, Waxman D (1998) Explaining the geographic distributions of sexual and asexual populations. *Nature* 391:889–892
- Porter ML, Crandall KA (2003) Lost along the way: the significance of evolution in reverse. *Trends Ecol Evol* 18:541–547
- Robinson DJ, Hall MJ (2002) Sound signalling in Orthoptera. *Adv Insect Physiol* 29:151–278
- Römer H (1983) Tonotopic organization of the auditory neuropile in the bushcricket *Tettigonia viridissima*. *Nature* 306:60–62

- Schumacher R (1973) Morphologische Untersuchungen der tibialen Tympanalorgane von neun einheimischen Laubheuschrecken. *Z Morphol Tiere* 75:267–282
- Schumacher R (1979) Zur funktionellen Morphologie des auditiven Systems der Laubheuschrecken (Orthoptera: Tettigoniidae). *Entomol Gener* 5:321–356
- Simmons LW, Bailey WJ (1990) Resource influenced sex roles of zaprochiline tettigoniids (Orthoptera: Tettigoniidae). *Evolution* 44:1853–1868
- Sinervo B, Calsbeek R (2003) Physiological epistasis, ontogenetic conflict and natural selection on physiology and life history. *Integr Comp Biol* 43:419–430
- Sismondo E (1980) Physical characteristics of the drumming of *Meconema thalassinum*. *J Insect Physiol* 26:209–212
- Stumpner A (1996) Tonotopic organization of the hearing organ in a bushcricket: physiological characterization and complete staining of auditory receptor cells. *Naturwissenschaften* 83:81–84
- Stumpner A, Heller K-G (1992) Morphological and physiological differences of the auditory system in three related bushcrickets (Orthoptera: Phaneropteridae, *Poecilimon*). *Physiol Entomol* 17:73–80
- Stumpner A, von Helversen D (2001) Evolution and function of auditory systems in insects. *Naturwissenschaften* 88:159–170
- Suomalainen E, Saura A, Lokka J (1987) Cytology and evolution in parthenogenesis. CRC Press, Florida
- Swallow JG, Garland T (2005) Selection experiments as a tool in evolutionary and comparative physiology: insights into complex traits—an introduction to the symposium. *Integr Comp Biol* 45:387–390
- Warchalowska-Sliwa E, Bugrov AG, Maryanska-Nadachowska A (1996) Karyotypes and C-banding patterns of some species of Phaneropterinae (Orthoptera, Tettigoniidae). *Folia Biol Krakow* 44:5–10
- West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, Oxford
- Wiens JJ (2001) Widespread loss of sexually selected traits: how the peacock lost its spots. *Trends Ecol Evol* 16:517–523
- Wilkens H, Culver DC, Humphreys WF (2000) Subterranean ecosystems. *Ecosystems of the world*, vol 30. Elsevier, Amsterdam
- Yager DD (1999) Structure, development and evolution of insect auditory systems. *Microsc Res Techn* 47:380–400
- Zuk M, Kolluru GR (1998) Exploitation of sexual signals by predators and parasitoids. *Quart Rev Biol* 73:415–438