Diversity of wing patterns and abdomen-generated substrate sounds in 3 European scorpionfly species

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Abstract  In the genus Panorpa (Insecta: Mecoptera), also known as scorpionflies, pre-mating behavior includes repeated sequences of slow wing movements (waving, fanning, flagging) which are accompanied by rapid abdomen vibrations that generate substantial substrate-borne sound. It is still unknown whether wing patterns or vibratory signals contain information about species identity, sex and/or the quality of potential mating partners. Besides species-specific pheromones, these multimodal signals may be of particular importance for the maintenance of reproductive isolation in sympatrically occurring scorpionfly species. Here, we analyzed phylogenetic relationships among, and the pattern of forewings as well as substrate-borne sound in 3 different sympatric Central-European scorpionfly species (P. communis, P. germanica, and P. alpina). Divergence time estimates, based on 879 bp of the mitochondrial COI gene, indicate longstanding separate evolutionary histories for the studied Panorpa species. Morphological analysis revealed that wing length as an indicator of body size increased in the following order: P. alpina < P. germanica < P. communis. Individuals can be assigned to the correct species and sex with high accuracy just by evaluation of the number of dark spots and the proportion of wing pigmentation. Despite high variability of interpulse period at an individual level, across species analysis revealed a positive correlation of average interpulse period as well as mean signal amplitude with forewing length. These results suggest wing patterns, but less likely vibratory signals, to contain information about species identity. Furthermore, receivers may be able to estimate the body size of a signaler solely on the basis of substrate-borne sound.

Key words  courtship signals; laser vibrometry; phylogeny; species recognition; substrate-borne sound; wing pattern

Introduction

It is commonly accepted that elaborate courtship behaviors evolved to exchange information about potential mating partners (Andersson, 1994). In the genus Panorpa courtship displays of males are multimodal and consist of visual, olfactory, and vibratory signals that are embedded in a ritual that may last for hours (Steiner, 1929; Rupprecht, 1974; Engqvist & Sauer, 2003; Engqvist, 2009). In many species males release pheromones and offer nuptial gifts in the form of saliva secretions and nuptial prey (usually dead arthropods). Such costly nuptial gifts may lead to the monopolization of resources by males of high quality. Therefore, Panorpa scorpionflies are frequently mentioned as an example of resource defense polygyny (Thornhill, 1981; but see Missoweit & Sauer, 2007). Mating success of females increases with the size of nuptial gifts offered by males (Sauer et al., 1998; for an example in bush crickets see Reinhold, 1999) due to
a tight correlation between the duration of copulation and the number of transferred sperm (Sauer et al., 1998; Engqvist & Sauer, 2001). Generally, costly signals such as nuptial gifts are believed to be honest indicators of a male’s quality since males of low quality may not afford the energy and time that is required to provide them (Zahavi, 1975).

Prior to offering costly nuptial gifts, male and female scorpionflies perform an elaborate ritualized pre-mating behavior (Engqvist & Sauer, 2002), including slow wing movements which is accompanied by brief sequences of rapid abdomen vibrations generating characteristic substrate-borne sound (see Fig. 1B; Steiner, 1929; Rupprecht, 1974). Substrate vibrations of scorpionflies may carry information about species identity, sex, and the readiness to mate (Rupprecht, 1974). Generally, vibratory signals produced in a mating context are most often species-specific and support mate localization (reviewed in Cokl & Virant-Doberlet, 2003; Hill, 2009; Orthoptera: Loher & Dambach, 1989; Plectoptera: Stewart, 1997; Sternorrhyncha: Kanmiya & Sonobe, 2002; Auchenorrhyncha: Claridge, 1985; Heteroptera: Cokl, 1983; Wilcox, 1995; Cokl et al., 2000; Neuropteroidea: Henry, 1994; Devetak, 1998; Coleoptera: Birch & Keenlyside, 1991; Hirschberger, 2001; Mecoptera: Rupprecht, 1974). In Panorpa species, the function of substrate-borne vibrations is still unclear and was suggested to synchronize long lasting courtship because imitation of conspecific vibrations elicits wing fanning and abdomen vibrations in dormant females (Rupprecht, 1974).

In Central Europe 5 different Panorpa species can be discriminated based on species-specific morphological criteria: Panorpa germanica L., P. communis L., and P. vulgaris IMH. LABR. P. cognate RAMB. P. cognata RAMB (Gepp, 1979). Whereas P. germanica, P. communis, P. vulgaris, and P. cognata belong to an exclusive Eurasian clade within the Panorpidae, P. alpina is clearly distinct, with some indication that it is more closely related to the North American representatives of this genus (Misof et al., 2000). Yet, nothing is known about divergence times within the genus Panorpa. Furthermore, it is still unclear whether wing pattern and substrate-borne sound generated by spontaneous abdomen vibrations contain information about species identity in sympatrically occurring Panorpa species where the information contained in these signals may be important for maintaining a prezygotic hybridization barrier.

In this study we investigated the degree of wing pigmentation as well as substrate-borne sound induced by spontaneous abdomen movements in the 3 sympatrically occurring Central-European Panorpa species: P. communis, P. germanica, and P. alpina.

Materials and methods

Animals and experimental protocol

Most scorpionflies used in this study were caught in the year 2009 in a habitat that is called “Sandhang” and is located at the border between Austria and Slovenia near Spielfeld (N 46°42′, E 15°37′). There, P. communis, P. germanica, and P. alpina live in sympatry. Additionally, some individuals were caught in the vicinity of Graz (Lustbühel: N 47°03′, E 15°29′), Sicheldorf (N 46°40′, E 16°1′), Tobisegg (N 46°54′, E 15°21′), and in a chasm (Deutschlandsberger Klause, N 46°48′, E 15°12′). Despite extensive search at these locations, only 1 individual with wing patterns typical of P. vulgaris and P. cognata was caught. In order to prevent an influence of multiple generations of scorpionflies within a season on the analyses (Engqvist, 2007), only individuals from the first generation were used in this study. After recording behavior and substrate vibrations generated by isolated scorpionflies on a film (wrapping film) with the help of a video camera and a laser vibrometer, insects were sacrificed with Chlorethylene and the right forewing was cut off for morphometric analysis. Additionally, scorpionflies were stored in pure alcohol for molecular phylogenetic analysis.

Wing morphometry

Right forewings of 128 scorpionflies belonging to 3 different species (P. alpina: 36; P. germanica: 21; P. communis: 71 individuals) were photographed with an ocular camera (DCM 510, 5 Megapixel OCS.tec Inc., Erding, Germany) mounted on a stereo microscope (Leitz, Germany). The number of dark spots, the area of dark wing pigmentation, wing length, and total wing area was analyzed after setting a manual threshold in the image analysis software ImageJ (NIH Image Version 1.43.67, USA). Images (see examples in Fig. 1C) were spatially calibrated using a calibrated micrometer. Parameters describing wing morphology can be used to assign wings to a certain species and sex. In a trial an error approach it turned out that the number of dark spots in combination with the degree of wing pigmentation allows correct classification of wings in most cases. Upper and lower limits of these parameters yielding the highest number of correct assignments were found using a nonlinear optimization method based on a generalized reduction gradient (Microsoft Excel-Solver, Leon Lasdon, University of Texas/Austin, and Allan Waren, Cleveland State University). Optimized limits used for assigning wings to a species and sex are shown in Table 1.
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Fig. 1 Experimental setup, premating behavior and wing morphology. (A) Setup for measuring spontaneous abdomen vibrations produced on a thin film. The beam of the laser vibrometer is focused on a reflective surface mounted in the middle of the film. (B) Schematic drawing of typical premating behavior: wing flagging and abdomen vibrations. Oscillograms of substrate-borne sound generated by abdomen vibrations and by walking over the substrate. (C) Wing patterns on right forewings of 3 sympatric Panorpa species that are subject of this study.

Table 1 Limits of criteria used to assign individual wings to 1 of 3 Panorpa species.

| Species and sex | Minimum number of dark spots | Maximum number of dark spots | Minimum proportion of dark pigmentation (%) | Maximum proportion of dark pigmentation (%) |
|-----------------|-----------------------------|-----------------------------|--------------------------------------------|--------------------------------------------|
| *P. alpina* male | 4.3                         | 10.6                        | 7.6                                        | 25.6                                       |
| *P. alpina* female | 7.6                        | 12.0                        | 13.8                                       | 24.9                                       |
| *P. germanica* male | 8.0                        | 11.7                        | 15.3                                       | 31.9                                       |
| *P. germanica* female | 9.2                        | 12.3                        | 23.5                                       | 34.1                                       |
| *P. communis* male | 4.9                         | 8.2                         | 29.1                                       | 46.4                                       |
| *P. communis* female | 5.6                        | 8.9                         | 31.1                                       | 44.8                                       |

Measurement of vibratory signals

Recording vibrations spontaneously generated by scorpionflies on a natural substrate are very difficult to obtain, which is due to the fact that these insects frequently change position. Therefore, we recorded vibrations on a transparent film (wrapping film) sealing the aperture of a coffee cup containing a single scorpionfly. Since scorpionflies show a positive phototactic response, it was possible to attract individuals to the underside of the film by bright...
light presented from above (see Fig. 1A). By combining video recordings (HDR-HC7, Sony Inc., Tokyo, Japan) with Laser–Doppler–vibrometry (PDV 100, Polytec Incorporation, Waldbronn, Germany) it was possible to discriminate sequences of substrate-borne sound generated by a vibrating abdomen from those vibrations of high amplitude generated in the course of a walking sequence. Vibrations are usually generated without direct contact of abdomen with the substrate. The analogue output of the laser vibrometer operating at a sampling rate of 20 kHz was A/D converted using a Powerlab (AD Instruments, Germany).

To maintain constant experimental conditions, the tension of the film was kept at a similar level. This was achieved with the help of a circle that outlines the aperture of the coffee cup (Fig. 1A). A similar setup was already used to record vibration signals of cryptic species belonging to the Chrysoperla carnea complex (lacewings, Henry et al., 2002). A reflecting tape was mounted in the middle of the film to increase the intensity of reflected laser light. External sound can cause vibration artifacts masking rather faint substrate-borne sound that is induced by abdomen vibrations. Therefore, all experiments were performed in a sound chamber located in the basement of the Institute of Zoology, Karl-Franzens-University of Graz. Ground vibrations were attenuated by performing all measurements on a heavy wooden table positioned on 4 air-filled balls. Ambient temperature inside this room was 20 ± 1 °C.

Interpulse period of abdomen-induced vibration pulses was analyzed by measuring the time span from one velocity maximum to the next. A time span of 350 ms was defined as the minimum interval that separates trains of pulses generated in close succession. Between 48 and 112 sequences related to abdomen vibrations were analyzed per species and sex. With the exception of P. alpina males, of which only 6 individuals produced abdomen vibrations, at least 10 individuals contributed to data in all groups (P. alpina females: 11, P. germanica males: 13, P. germanica females: 10, P. communis males: 15, P. communis females: 10 individuals). The following temporal parameters describing sequences of vibration pulses were manually evaluated in Chart (Version 5, AD Instruments): number of pulses, average pulse period, minimal and maximum pulse period. Frequency analysis of vibratory signals was performed in Chart by using an FFT analysis based on 512 points.

DNA data

Inference of phylogenetic relationships and tentative divergence times among the studied taxa was based on 879 bp of the mitochondrial Cytochrome Oxidase I (COI) gene. Whole genomic DNA was extracted from thorax musculature (6 P. alpina, 14 P. communis, and 15 P. germanica) preserved in 96% ethanol by proteinase K digestion followed by protein precipitation with ammonium acetate. PCR amplification of 2 fragments of the COI gene using the primers C1-J-1751 and C1-N-2191 (fragment 1), and C1-J-2165, and T2L-N-3014 (fragment 2) (Simon et al., 1994; Misof et al., 2000), followed the protocol of Koblmutlter et al. (2011), but with an annealing temperature of 50 °C. PCR-products were purified with ExoSAP-IT (Amersham Biociences, Freiburg, Germany) prior to being added as template for chain termination sequencing following the protocol in Duftner et al. (2005). DNA fragments were purified with Sephadex TM G-50 (Amersham Biociences) and visualized on an ABI 3130xl capillary sequencer (Applied Biosystems). Electropherograms were checked and edited and alignments were conducted in MEGA5.1 (Tamura et al., 2011). Sequences are deposited in GenBank under the numbers KJ746063-KJ746097.

Haplotype (Hd) and nucleotide diversity (π) were estimated in DnaSP 5.10 (Librado & Rozas, 2009). The most appropriate substitution model (T92+G, Tamura, 1992) was selected based on the Bayesian Information Criterion in MEGA5.1. Phylogenetic analysis was conducted by means of Bayesian Inference (BI) in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Posterior probabilities were obtained from a Metropolis-coupled Markov chain Monte Carlo simulation (2 independent runs; 8 chains with 3 million generations each; trees sampled every 1 000 generations; the first 25% of trees were discarded as burn-in). Chain stationarity and run parameter convergence were checked in Tracer 1.5 (Rambaut & Drummond, 2009; all ESS > 200). Mean divergence between clades inferred in MEGA5.1 were translated into tentative absolute age estimates employing published minimum and maximum insect COI substitution rates of 0.0075 and 0.0175 substitutions/site/million years, respectively (Farrell, 2001; Papadopoulou et al., 2010).

Statistics

Except phylogenetic analysis, we performed all statistical calculations in Sigmaplot 12.3 (Systat Software Inc., Germany). Multigroup comparison was performed by application of an ANOVA. Data was checked for normal distribution using a Shapiro–Wilkinson test. A correlation between parameter pairs was tested for significance by means of a Spearman’s rank order correlation.

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Results

Wing patterns

In scorpionflies forewing length correlates with body size (Thornhill & Sauer, 1992) and shows only little intraspecific variation in the first generation of a year (coefficient of variation of wing length = 3%–6%). In a between species comparison wing length and total wing area increases in the following order: \textit{P. alpina} < \textit{P. germanica} < \textit{P. communis} (average wing length: \textit{P. alpina} males: 11.8 ± 0.7 mm; \textit{P. alpina} females: 12.2 ± 0.5 mm; \textit{P. germanica} males: 12.2 ± 0.4 mm; \textit{P. germanica} females: 13.2 ± 0.4 mm; \textit{P. communis} males: 14.2 ± 0.6 mm; \textit{P. communis} females: 15.1 ± 0.8 mm). Sexual dimorphism is evident in wing morphology as wing area of females is higher compared to their male counterparts (Fig. 2A, gray bars). On average wing area of \textit{P. communis} females is significantly higher than wing area of other species \((P < 0.05, \text{Kruskal–Wallis One Way ANOVA on ranks followed by a Holm–Sidak post hoc test})\). Wings of \textit{P. communis} males are on average larger than any other species and sex with exception of \textit{P. germanica} females species \((P < 0.05, \text{Kruskal–Wallis One Way ANOVA on ranks followed by a Holm–Sidak post hoc test})\). Between species comparison shows that the proportion of dark wing pigmentation significantly increases with wing area (Fig. 2A, \(P < 0.001, \text{correlation coefficient} = 0.79, n = 121, \text{Spearman’s rank order correlation})\). \textit{Panorpa germanica} wings exhibit the highest number of dark spots and \textit{P. communis} the fewest (Fig. 2B). Therefore, dark spots on \textit{P. communis} wings are larger compared to the spotted pigmentation of \textit{P. alpina} and \textit{P. germanica}. Interspecific differences in the number of dark spots and the degree of wing pigmentation allowed correct assignment of individual wings to a species and sex in 75%–90% of cases (Fig. 2C). Based on the criteria shown in Table 1 individuals of \textit{P. communis} were never falsely classified. In \textit{P. germanica} and \textit{P. alpina} false classification was evident in only 3%–14% of cases.

Vibratory signals

Abdomen vibrations induce a train of substrate vibrations consisting of individual pulses of variable amplitude with an average maximum velocity of 1.04 ± 0.37 mm/s. The average duration of pulse trains as well as the average number of pulses comprising a tremulation sequence was not different between species and sex \((P > 0.05, \text{Kruskal–Wallis ANOVA on ranks})\). Although individual variability of interpulse period was high (coefficient of variation = 18%–25%), mean interpulse period of \textit{P. alpina} was significantly shorter compared to \textit{P. communis} females \((P < 0.05, \text{Kruskal–Wallis One Way ANOVA on ranks followed by a Holm–Sidak post hoc test})\). In contrast, interpulse periods of substrate vibrations generated by \textit{P. germanica} males and females were not
different from any other species. Across species comparison revealed a positive correlation between individual forewing length and mean interpulse period ($P < 0.001$, correlation coefficient $= 0.73$, Spearman’s rank order correlation; see Fig. 3B) as well as a positive correlation between this body size-related parameter with the average maximum vibration velocity of individuals ($P < 0.05$, correlation coefficient $= 0.57$, $n = 24$, Spearman’s rank order correlation). However, larger individuals tend to produce fewer vibration pulses in a sequence ($P < 0.05$, correlation coefficient $= -0.531$, $n = 24$, Spearman’s rank order correlation). Spectral analysis of abdomen-induced vibrations generated on the film exhibited energy peaks in the range between 300 and 500 Hz. Dominant carrier frequencies were not significant between species ($P > 0.05$, Kruskal–Wallis One Way ANOVA on ranks followed by a Holm–Sidak post hoc test).

Phylogenetic relationships and divergence times

Phylogenetic inference based on partial COI sequences of 35 individuals yielded 3 distinct genetic clusters corresponding to the 3 study species, with $P. alpina$ representing the most distantly related taxon (Fig. 4, Table 2). Haplotype diversity ($H_d$) and nucleotide diversity ($\pi$) were 0.867 and 0.00334 for $P. alpina$, 0.923 and 0.00975 for $P. communis$, and 0.962 and 0.00534 for $P. germanica$, respectively. However, these values need to be interpreted with caution, as sample size was quite low, especially for $P. alpina$. Estimated interclade distances (Table 2) translate into divergence times of 4.9–11.4 MYA for the split between $P. communis$ and $P. germanica$ and 7.5–17.6 MYA for the split between $P. alpina$ and $P. communis$ + $P. germanica$. Although morphologically unambiguously identified as $P. communis$, 5 individuals had a COI haplotype identical or similar to a previously published sequence of $P. vulgaris$ (Misof et al., 2000).

Discussion

A high individual variability of the pulse period, the number of pulses and sequence duration of substrate-borne sound generated by abdomen vibrations questions these signal parameters to contain relevant information about species identity. Therefore, scorpionflies may not be able to recognize conspecifics solely by evaluating the temporal structure of vibratory signals, which does not exclude the possibility that species information is contained in the carrier frequency of substrate-borne sound (for an example in stink bugs see Cokl, 1983). In the study of Rupprecht (1974) the dominant frequency of vibratory signals of $P. alpina$, $P. communis$, and $P. germanica$ was $\approx 100$ Hz and in some rare cases $\sim 200$ Hz in $P. communis$. A rather high dominant frequency of vibratory signals of about 400 Hz obtained in this study is most likely the result of resonance properties of the substrate (film). Preliminary experiments performed on a stinging nettle ($Urtica dioica$) showed that substrate-borne vibrations generated there exhibit a dominant carrier frequency of only $\sim 40$ Hz. Since scorpionflies are abundant on various substrates with
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Fig. 4  Phylogenetic relationships (BI tree inferred in MrBayes 3.1.2) of the 3 studied Panorpa species based on 879 bp of the mitochondrial COI gene. Only posterior probabilities >0.7 are shown. Different colors refer to different sampling localities. Asterisks indicate individuals morphologically identified as P. communis, but genetically identical or closely related to previously published sequences of P. vulgaris (Misof et al., 2000).

In contrast to abdomen induced vibrations, different patterns and degrees of wing pigmentation in combination with species-specific pheromones produced by all 3 Panorpa species (Bornemissza, 1964; Thornhill, 1973, 1979, 1992; Kovalev & Nikolaeva, 1989; Thornhill & Sauer, 1992; Kock et al., 2007) may function as prezygotic barriers against interspecific gene flow between sympatric scorpionfly species. However, the long-standing separate evolutionary history (in sympatry) of the European Panorpa species points to an early evolution of important, potentially multimodal signals for preventing interspecific gene flow. Molecular diversity indices indicate (despite small sample sizes) that effective population sizes in the studied region have been constant and large in the recent history of the species. The genetic similarity (at least in mtDNA) between P. vulgaris and P. communis (this study; Misof et al., 2000) suggests introgressive hybridization and/or incomplete lineage sorting in these sister species.

Substrate-borne vibrations recorded in this study are the byproduct of a rather conspicuous visual signal namely a vibrating abdomen with a reddish tip. Therefore, substrate-borne vibrations have to be regarded as a fixed signal component of a rather conspicuous visual signal (Smith, 1977; Partan & Marler, 2005). Generally, multiple cues increase the amount of information gained by receivers (e.g., Candolin & Reynolds, 2001) and facilitate mate assessment under different environmental conditions (Reynolds, 1993; Endler & Houde, 1995). Furthermore, multimodal signaling aids receivers in detecting and locating the source of the signal (Latimer & Schatral, 1983; Rowe, 1999; Partan & Marler, 2005; Higham & Hebets, 2013). From the literature about multimodal communication it can be deduced that substrate-borne vibrations generated by scorpionflies likely improve detection of a

Table 2  Mean uncorrected and corrected (T92+G model of molecular evolution; Tamura, 1992) interclade distances.

| Combination                        | Mean uncorrected distance | Mean corrected (T92+G) distance |
|------------------------------------|---------------------------|--------------------------------|
| P. communis/P. germanica           | 0.0826                    | 0.1706                         |
| P. alpina/P. communis              | 0.1050                    | 0.2746                         |
| P. alpina/P. germanica             | 0.1001                    | 0.2528                         |
| P. alpina/P. communis + P. germanica | 0.1025              | 0.2633                         |

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corresponding visual stimulus signaling the readiness to mate (Rupprecht, 1974) as well as the body size of the potential mate.

*P. communis* is often found courting during daytime, which suggests a rather high degree of wing pigmentation to aid species identification (Sauer & Hensle, 1979). However, species recognition on the basis of wing pigmentation is likely restricted to a rather short sender-receiver distance as the consequence of a weak spatial resolution of compound eyes. Assuming a realistic spatial resolution of about 1° (Kirschfeld, 1976), a scorpionfly has to be located 2.8 cm next to another individual to detect a spot with a diameter of 0.5 mm and within 11 cm to detect the dark strip of a *P. communis* individual. Given rather sensitive vibration receptors of arthropods (Devetak et al., 1978; Markl, 1983), substrate-borne vibrations with an average maximum amplitude of about 1 mm/s may be detectable at a distance of about 1–2 m (compare with Rodriguez et al., 2004; Cokl et al., 2007; Römer et al., 2010; Eriksson et al., 2011), whereby the dimension of the substrate does not necessarily limit signal transmission (Eriksson et al., 2011; Kavcic et al., 2013). In an open habitat receivers likely detect wing movements, a vibrating colored abdomen and coherent substrate-borne vibrations at some distance from the signaler. Nevertheless, receivers have to be in close proximity to extract information about species identity and sex solely on the basis of visual and vibratory cues. In contrast, pheromones released by males of all 3 species have to be regarded as long distance communication signals enabling species identification by means of a specific cocktail of volatiles (Thornhill, 1973, 1979, 1992; Gerhards, 1999).

If visual signals are not available, information about body size is roughly encoded in the average interpulse period of vibration pulses. Signaling body size not just plays an important role during courtship but also in aggressive encounters (Thornhill, 1984; Sauer et al., 1998). As long as energetic demands associated with the generation of vibratory signals in scorpionflies are unknown, it remains speculative whether this signal is prone to cheating or not (Zahavi, 1975). However, cheating body size will be uncovered in the course of physical encounters (Sauer et al., 1998) as well as during long lasting courtship where potential mating partners assess body size using antenna and by evaluating visual cues. Theoretically, wing length and wing patterns offer receivers the possibility to evaluate fluctuating asymmetry as was suggested as a quality indicator in a Japanese scorpionfly species (Thornhill, 1992). Meanwhile, the importance of fluctuating asymmetry in the context of mate choice was questioned; instead a correlation of this parameter with other male traits, indicating quality more reliably, is likely (Swaddle, 2003).

In all 3 species, forewings of females exhibit the more conspicuous wing pattern, which is reflected in a higher degree of wing pigmentation (Fig. 2B). This indicates a sex-role reversal (Gwynne, 2008) in these *Panorpa* species where males are choosy due to the provision of costly nuptial gifts, such as salivary gland secretions (Engqvist & Sauer, 2002) or the risky acquisition of dead arthropods (Thornhill, 1975). In addition to the costs associated with nuptial gifts, the choosiness of a potential mating partner may strongly depend on the operational sex ratio (OSR) (Gwynne, 1990). Field catches made in the course of this study did not indicate a higher abundance of one sex over the other in all 3 *Panorpa* species though sample sizes were rather small. Therefore, OSR is unlikely to play a key role in mate choice decisions in the species that are subject of this study.

To conclude, this study demonstrates the absence of species-specific information in substrate-borne sound that is generated as a fixed signal component of rapid abdomen vibrations, which suggests this multimodal signal to facilitate detection of mating displays containing information about the readiness to mate and the quality of signalers. The latter is obvious in a positive correlation between forewing length (body size) and average pulse period of substrate vibrations as well as in the positive correlation of body size and maximum vibration amplitude. In contrast to the information conveyed in vibration signals, only 2 parameters describing wing ornaments were sufficient to successfully assign individual wings to 1 of 3 *Panorpa* species (except *P. vulgaris*). Nonetheless, a weak spatial resolution of compound eyes of insects suggests a limited detection range of species-specific wing patterns. Additional experiments on a natural substrate are needed to gain further insights into the function, reliability, and information content of multimodal signals in this fascinating insect order.

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Disclosure

All authors declare no potential conflicts of interest including specific financial interests, relationships and affiliations.

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