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# Agonistic signals received by an arthropod filiform hair allude to the prevalence of near-field sound communication

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**Abstract**
Arthropod filiform hairs respond to air particle movements and are among the most sensitive animal sensory organs. In many species, they are tuned to detect predators or prey and trigger escape or prey capture behaviours. Here we show for the first time that these hairs also receive intraspecific near-field sound signals in an arachnid. During agonistic encounters, whip spiders (Arachnida, Amblypygi) perform antenniform leg vibration (ALV) displays that have significantly longer duration in contest winners than losers. During an ALV display: (i) the vibrating antenniform leg of the displaying whip spider is positioned close to the trichobothria (filiform hairs) on its opponent's walking legs, (ii) the vibrating antenniform leg can excite these trichobothria via air movements and without direct contact, (iii) the antenniform leg of the displaying whip spider vibrates at a frequency that causes particularly strong, sustained excitation and little adaptation in the trichobothria, and (iv) the duration of an ALV display can be extracted from the response of a trichobothrium. Since filiform hairs are widespread among arthropods, communication via such hairs could be extremely prevalent.

**Keywords:** communication, trichobothria, signal-receiver match, amblypygid, arachnid, whip spider

## 1. Introduction

Arthropod filiform hairs detect air (or water) particle displacements (Gorner & Andrews 1969) and are among the most sensitive animal sensory organs (Shimozawa *et al.* 2003). Such near-field particle movements are used in intraspecific communication by a restricted group of insects, but these use specialized organs for particle movement detection, such as the Johnston's organ of some insect antennae (e.g. Johnston 1855; Tauber & Eberl 2003; Gibson & Russell 2006; Tsujiuchi *et al.* 2007). Whether filiform hairs also receive such intraspecific signals is unclear, but given their abundance across different arthropod taxa, their use in signal reception may indicate that near-field souads are a far more common communicatory modality than previously realized.

Insect filiform hairs and arachnid trichobothria (analogous, but not homologous, sensilla) function in the detection and evasion of predators and their response properties are often tuned to this role (e.g. Camhi *et al.* 1978; Tautz & Mark1 1978; Igelmund & Wendler 1991; Gnatzy 1996; Suter 2003). For example, the population of filiform hairs on a cricket's cerci are tuned to the 120–200 Hz range of acoustic frequencies produced by flying insect predators (Magal *et al.* 2006). Likewise, arachnid trichobothria effectively detect prey movements and mediate prey capture behaviors (e.g. Beck & Gorke 1974; Barth & Holler 1999; Barth 2002). A possible role for these hairs in intraspecific communication is currently illustrated by only one example: in an African cave cricket lacking stridulatory organs, males produce silent wing flicks during courtship and aggressive encounters that induce air currents which excite the filiform hairs of females (e.g. Heidelbach *et al.* 1991). These are proposed to cause the receiving female to become more passive and less responsive to disturbing stimuli (Heidelbach & Dambach 1997), although it has not yet been shown that the effective component of this signal is air movement rather than visual, olfactory or vibration cues (Pollack *et al.* 1998). Nevertheless, since near-field sounds can be produced using limb movements, intraspecific signals of this sort could be widespread among animals possessing filiform hairs.

Here we investigate whether arachnid trichobothria function in intraspecific near-field sound detection during the agonistic contests of whip spiders (Arachnida, Amblypygi). Whip spiders encompass their own arachnid order and possess enlarged, spiny pedipalps, and modified (antenniform) first legs used as feelers rather than walking legs. Contests consist of a sequence of ritualized displays, the most striking of which is a rapid vibration of the displayer's outstretched antenniform leg close to its opponent (Alexander 1962; Weygoldt 2000; Fowler-Finn & Hebets 2006). In complete darkness (excluding visual detection), this display is performed for longer by contest winners than losers, and probably conveys information used to resolve contest outcome (Fowler-Finn & Hebets 2006).

We show that the agonistic antenniform leg vibrations (ALVs) of the whip spider *Phrynus marginemaculatus* are received by trichobothria and are suited to eliciting a particularly strong response in them. During ALVs, the vibrating antenniform leg is positioned close to the receiver's trichobothria; ALV excites these trichobothria via near-field sound and occurs at a frequency causing an especially strong and sustained response in them. As a result, ALV duration can be extracted from the trichobothria response. This is the first reported match between an intraspecific near-field sound signal and the response properties of a filiform hair. We suggest that such near-field sound communication may be more common across arthropod species possessing filiform hairs than previously recognized.

## 2. Material and Methods

Male *P. marginemaculatus* were collected from Big Pine Key, FL, USA, in August 2006 and housed on a reversed 12 : 12 hours light cycle. Experiments were performed at 23–24°C during the subjective night.

### (a) *Investigating production and reception of ALV*

We staged 21 agonistic encounters between eight mature male whip spiders. Each animal was paired in multiple contests with previously unencountered opponents. Contests were separated by more than 8 days to exclude stress or fatigue effects.

Contests were staged in a 20 cm diameter circular arena with 5 cm high acetate sides. Lighting was from standard fluorescent room lights and an infrared LED source (StroboLED v3, AOS technologies, Baden, Switzerland) to allow filming; we noted no effects of lighting on contest behavior. Each contest was filmed at low speed from three angles (front, back and top) using mirrors and a horizontally mounted digital camcorder (DCR-HC65, Sony Electronics, Inc., USA). An ALV display from each animal was also filmed simultaneously at 500FPS using a Fastcam 1024PCI high-speed digital camera (Photron USA, San Diego, CA, USA) mounted next to the camcorder. The short high-speed recording length, and time taken to write captured video to disk, prevented us from filming ALVs of both individuals in a contest.

We used low-speed films to measure contest phases observed, contest duration and ALV duration. We used high-speed films to analyze the period and amplitude of antenniform leg movement during ALV and the location of the display relative to the receiver (electronic supplementary material).

### (b) *Investigating sensory cell responses to ALVs*

Trichobothrium recordings were made from the eight males used in behavioral experiments (after more than four weeks), plus two males that matured during those experiments.

A walking leg patella has two trichobothria (termed Pa1 and Pa2; §3). We recorded from both leg 3 trichobothria in separate experiments. Whip spiders were fixed in a standardized standing position using plasticene and insect pins (leg 3 tibia flexed 90° to femur and 45° to horizontal). Recordings were made using two 50 μm copper wires insulated but for their tips (Advent Research Materials Ltd, Eynsham, UK), inserted through the wallzing leg femur. Electrode wires did not interfere with trichobothria movements. Recordings were differentially amplified using a standard AC amplifier and captured to disk using a power 1401 A-to-D converter and SPIKE2 v.5 for Windows (Cambridge Electronic Design Ltd, Cambridge, UK).

Only the trichobothrium under investigation in a particular experiment was recorded: the other patella trichobothrium was removed and the tibia, tarsus, and metatarsus of leg 3 were tightly covered with aluminium foil. Manual stimulation of the hair, and comparison with responses to directed air puffs, identified the trichobothrium being recorded.

We could not measure the airflows induced by ALVs, so to reproduce them we used a stimulator (after Widmer *et al.* 2005) that vibrated a 200 μm diameter tungsten wire. Since the back and forth movements of the antenniform leg during ALV are a dipole source, moving this similarly sized wire in the same way should induce the same air movements. Briefly, sine wave voltages moved the voice coil of a vertically mounted mini speaker in the horizontal plane. Attached perpendicular to the voice coil was a tungsten wire bent at 90° to leave a 27 mm horizontal portion of wire that moved from side to side with movements of the speaker voice coil. The entire stimulator, excluding only the horizontal wire portion, was shielded from the preparation so that excitation came from the side-to-side movement of the wire only (supplementary material). This was confirmed in control experiments (supplementary material). Speaker movements were measured using an infrared LED-photodiode pair.

We used stimulus vibration frequencies of 1–120 Hz. Oscillation of the stimulus wire tip had a mean amplitude of 2600 μm (measured using a high-speed camera and dissecting microscope). Individual stimulus amplitude varied from this mean by up to 150 μm, due to flexions of the tungsten wire that we could not control. These variations did not explain overall patterns in our data (e.g. figure 3), and were within the range seen during whip spider ALV. The tungsten wire was positioned from anterior to posterior over the trichobothrium under investigation—30° angle to tibia, 1 mm above the trichobothrium cup and protruding 1 mm past it posteriorly—using a micromanipulator. Stimuli had 5, 20, or 90 s durations and were delivered using a 120 s interval. Each trichobothrium was recorded in six whip spiders; each received four to six presentations of each stimulus.

Whip spiders are difficult to collect and recovered post-experiment. We did not sacrifice animals to make trichobothrium measurements, but one large adult male was sacrificed for reasons unconnected with this study. We made measurements of both patella trichobothria from its removed left leg 3 using a dissecting microscope, Spot Flex 64MP digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI, USA) and IMAGEPRO Discovery software (Media Cybernetics, Inc., Bethesda, MD, USA).

Unless otherwise stated, we quote means ± s.e.m.

## 3. Results

### (a) *Description of ALV display*

Whip spider contests staged in this study were typical of those described previously and ALV was observed in 73.7% (14) of contests (supplementary material). During ALV, the displaying whip spider extended one antenniform leg towards its opponent and rapidly vibrated it from side to side in the horizontal plane (figure 1a). ALV made no audible sound and was sometimes performed simultaneously by both competitors. The contest winners performed ALVs for a significantly greater fraction of total contest duration than the losers (one-tailed *t*-test against 0, $t = 2.86$,

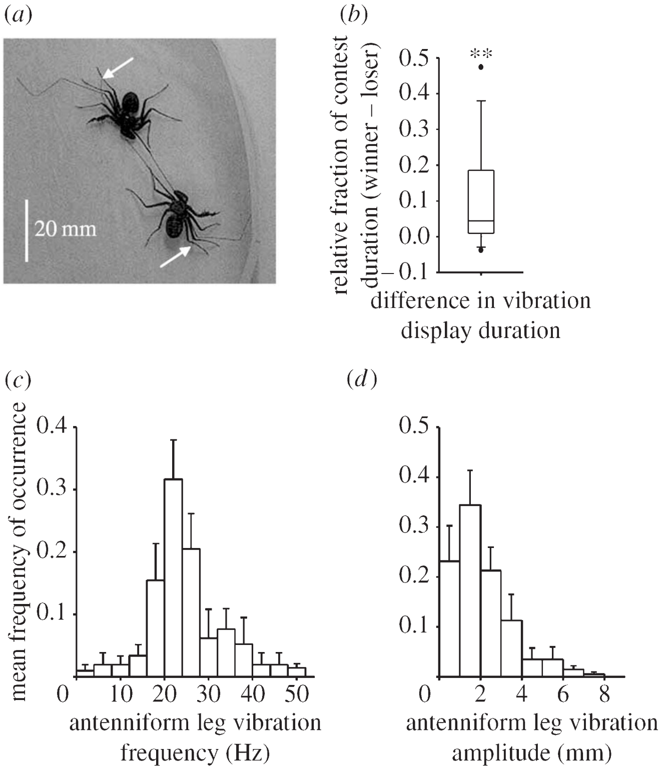

**Figure 1.** Whip spider ALV displays. (**a**) Frame from a high-speed recording of two males performing ALV. During ALV, one antenniform leg of the signaller is extended towards the receiver and rapidly vibrated. Arrows mark antenniform leg tips. (**b**) ALVs are produced for a greater fraction of contest duration by contest winners than losers. For 14 contests containing ALV, the fraction of contest duration that the loser performed ALV was subtracted from that the winner did. Box = 25th and 75th percentiles; bisecting line = median; whiskers = 10th and 90th percentiles; outliers plotted. Asterisks indicate data that are significantly greater ($p < 0.0\ 1$) than a test mean of zero (main text). (**c**) Mean frequency of occurrence of different vibration frequencies in ALVs from seven male whip spiders. Vibration periods were measured for 30 consecutive ALV cycles filmed at high speed and converted to frequency. Bins are 4 Hz; bars are s.e.m. (**d**) Mean ALV amplitude for 30 consecutive cycles of ALV recorded from seven male whip spiders in (c). Bins are 1 mm; bars are s.e.m.

$p = 0.0067$, d.f. = 13; figure 1b), as noted in a previous study conducted in complete darkness (Fowler-Finn & Hebets 2006). ALVs were produced for a mean total duration of 66.6 ± 13.1 s by the contest winners and 55.4 ± 13.8 s by the losers. These comprised bouts of vibration from 2 to 91 s in duration (mean = 20.7 ± 2.1 s).

We successfully high-speed filmed ALVs in seven whip spiders. The mean vibration cycle period was 46.75 ± 6.46 ms, corresponding to 21.4 Hz (figure 1c), and mean vibration amplitude was 2.15 ± 0.32 mm (figure 1 d). The antenniform leg always vibrated from side to side in the horizontal plane.

### (b) *Reception of ALV displays*

From the side, the signaller's antenniform leg arched downwards; only its tip came close to the receiver's rearmost leg or opisthosoma (abdomen). Sometimes the tip of the signaller's antenniform leg came into close proximity with the receiver and may have contacted it (we could not be certain of contact since the antenniform leg did not flex as during infrequent strong contact). The frequencies at which ALV cycles might have contacted the receiver for each animal were 0.00, 0.00, 0.41, 0.47, 0.48, 0.68, and 0.81. These possible contacts normally occurred with the tibia or patella of the receiver's rearmost legs, or with the side of its opisthosoma, but they appeared too irregular to be the primary means of ALV reception.

During ALV, the antenniform leg of a signaller was located above the receiver's proximal walking legs for the greatest proportion of display duration, often as little as 1 mm above them (figure 2a). We found two trichobothria on the dorsal and the anterior patellae of the walking legs that would be particularly close to an ALV display. We term these trichobothria Pa1 and Pa2, respectively (figure 2b). Pa1 is on the dorsal patella, approximately 280 μm from its distal margin. In an adult male whip spider, its length was 386 μm and it was easily deflected by air currents and almost constantly in motion during observation. Pa2 is more anterior than Pa1, approximately 100 μm from the patella's distal margin. In an adult male whip spider, its length was 193 μm and it was less easily stimulated than Pa1, requiring directed air currents. While standing, the dorsal surface of the leg is tilted posteriorly, so that the two trichobothria project rearwards (Pa1) and upwards (Pa2).

### (c) *Trichobothriurn responses to ALV*

Electrophysiological recordings showed that trichobothria Pa1 and Pa2 responded to direct contact (data not shown). We wanted to see whether ALV displays could also excite these trichobothria without contact via near-field sound. We simulated the air movements induced by ALV using a device that moved a wire of the same thickness as an antenniform leg at an equivalent amplitude and frequency to real ALV displays (§2; supplementary material). With this stimulus positioned as during ALV, without physical contact between stimulus and trichobothrium, we recorded action potentials in Pa1 and Pa2 in response to simulated ALV (figure 3a,b). We noted two action potentials from Pa1 and one from Pa2. These were phase-locked to the ALV stimulus across a range of vibration frequencies (from 1 to 120 Hz; supplementary material). However, higher simulated ALV frequencies caused faster adaptation of the responses of both trichobothria (figure 3c). Thus, the strongest response over time was induced by the highest simulated ALV frequency that did not induce strong adaptation, as indicated by total action potential numbers induced in each trichobothrium by each stimulus. These counts fitted a log-normal distribution around the most common frequencies in real ALVs for both Pa1 and Pa2 (figure 3d).

When stimulated with 90 s simulated ALVs (equal in length to the longest bouts observed) at 25 Hz, Pa1 mechanoreceptors adapted but remained responsive throughout stimulation (figure 4a). Repeated 90 or 20 s simulated ALV bouts separated by 5 s caused little habituation in Pa1 mechanoreceptors (figure 4b,c). These could signal ALV duration: when ten 20 s vibration bouts separated by a 5 s inter-stimulus interval were delivered to a single individual (figure 4c), the mean time between first and last action potential in each bout was 19.759 ± 0.068 s for the larger action potential and 19.934 ± 0.014 s for the smaller action potential, matching the 20 s stimulus duration.

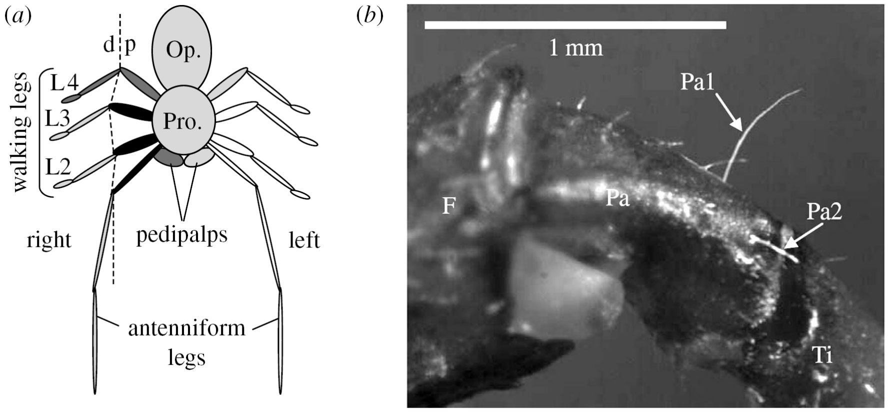

**Figure 2.** ALV occurs close to the walking leg trichobothria. (**a**) The mean proportion of ALV display duration that the signaller's antenniform leg overlays particular receiver body regions. Proportions of ALV duration are indicated by: white, 0.00; light grey, 0.01–0.25; dark grey, 0.26–0.50; black, greater than 0.51. The receiver is drawn from a dorsal aspect, and body regions are as follows: opisthosoma (or abdomen; Op.); prosoma (or cephalothorax; Pro.); pedipalps; proximal leg regions (p); and distal leg regions (d). Proportions of ALV duration were calculated by recording antenniform leg position at 10 ms intervals from 1600 ms samples of ALV for each of the seven whip spiders. Mean proportions of ALV duration and standard deviations for each body region are listed in the supplementary material. Data are corrected as if the signaller performed ALV with its left antenniform leg. (**b**) Trichobothria on the walking leg patella. Patella pictured from above a whip spider in normal standing position (leg dorsal aspect twisted posteriorly). F, femur; Pa, patella; Ti, tibia; Pa1 and Pa2, patella trichobothria 1 and 2.

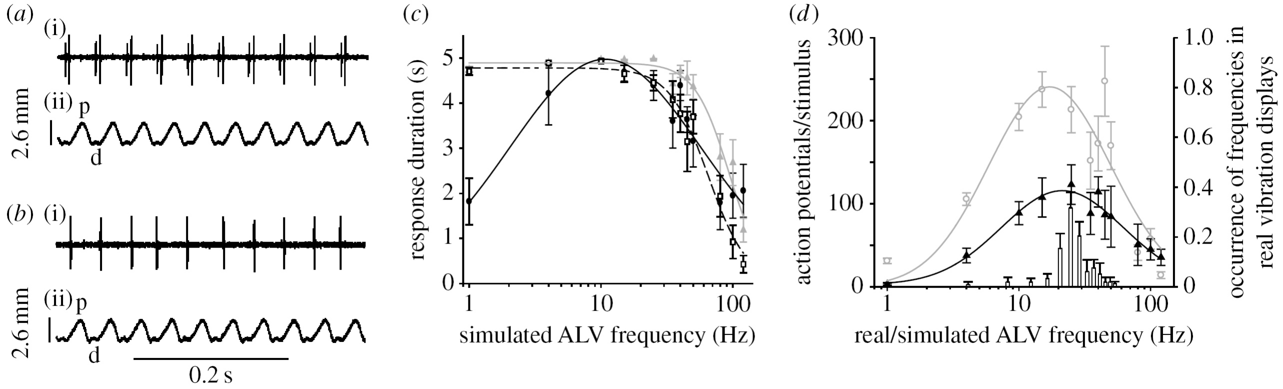

**Figure 3.** Simulated ALVs excite trichobothria Pa1 and Pa2 via air movements. (**a**) Simulated ALV at 25 Hz elicits two sizes of phase-locked action potential in recordings from Pa1. (i) Pa1 recording, (ii) stimulator movement (p, proximal; d, distal). (**b**) The same stimulation to Pa2 elicits one size of phase-locked action potential (AP). (i) Pa2 recording, (ii) stimulator movement. (**c**) Adaptation of the Pa1 and Pa2 responses to 5 s bursts of simulated ALV at varying frequency. Adaptation is measured as the mean time of the last AP in a response. Bars are s.e.m. and nonlinear regression lines are fitted (Pa1 small AP, grey triangles and grey line, $R^2$ = 0.95; Pa1 large AP, open squares and dashed line, $R^2$ = 0.97; Pa2 AP, black circles and black line, $R^2$ = 0.91). (**d**) Mean total AP numbers in Pa1 and Pa2 in response to 5 s simulated ALV at various frequencies. For Pa1, both sizes of AP are considered together. Log-normal curves were fitted by nonlinear regression (Pa1, grey circles and grey line, $R^2$ = 0.85; Pa2, black triangles and black line, $R^2$ = 0.73). Histogram re-plots the frequency of occurrence of vibration frequencies within real ALV displays (open bars) from figure 1c. Bars are s.e.m. Trichobothrium data are the means of four to six stimulus replicates to each of six male whip spiders in (c,d).

## 4. Discussion

We describe the agonistic ALVs of whip spiders. These displays are produced for longer by the contest winners than the losers and have previously been proposed to convey information used to resolve agonistic contests (Fowler-Finn & Hebets 2006). The form of an ALV display is suited to providing sustained, strong excitation to the two closest trichobothria on the walking leg patellae, and thus these trichobothria can convey ALV duration to the central nervous system. This is, to our knowledge, the first reported match between an intraspecific near-field sound signal and the response properties of a filiform hair.

ALVs are produced by whip spiders during agonistic contests, and for a significantly greater duration by the contest winners than the losers (Alexander 1962; Weygoldt 2000; Fowler-Finn & Hebets 2006). ALVs occurred in 73.7% of contests; normally one individual retreated following these, and only 10.5% of contests escalated to physical contact. This suggests that ALV displays are signals conveying information important for resolving contest outcome.

Our data suggest that agonistic ALVs are directed towards and received by the opponent's trichobothria. During ALV, the antenniform leg of the signaller is positioned close to the patella trichobothria of the receiver, indicating that they would be strongly stimulated by near-field air movements induced by ALV. Furthermore, the frequency at which the antenniform leg is vibrated during ALV causes the strongest sustained response in the trichobothria via near-field sound. This is because lower vibration frequencies cause less spikes per second (because spikes are phase-locked to the stimulus) and higher vibration frequencies cause the response to be curtailed by adaptation. As a result,

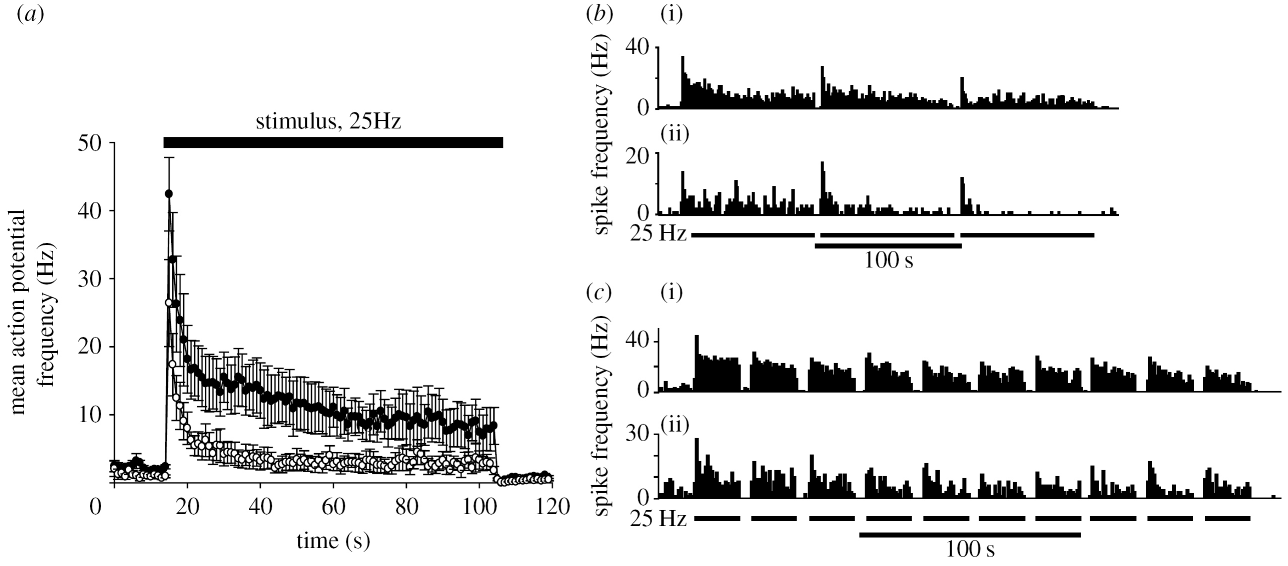

**Figure 4.** Stimulation of Pa1 with simulated ALVs representing aspects of real displays. (**a**) Peri-stimulus time histogram of Pa1 response to 90 s simulated ALV at 25 Hz. Data are plotted for both action potentials (AP) and are the mean of four stimulus presentations each to six male whip spiders. Bins are 1 s; bars are s.e.m. (filled circles, small AP; open circles, large AP). (**b, c**) Response of Pa1 mechanoreceptors in a single whip spider to repeated bouts of simulated ALV with a 5 s inter-stimulus interval. Bins are 1 s (i, small AP; ii, large AP).

the trichobothria can respond throughout simulated vibrations equal in length to the longest ALV observed during whip spider contests. Furthermore, real ALV displays were often composed of several vibration bouts. The lengths of simulated bouts, separated by seconds, could be extracted from the trichobothrium response. Therefore, these trichobothria are ideally suited to receiving the ALV signal and conveying its duration to the central nervous system. The match between signal and trichobothrium response is good evidence that the trichobothria are the intended target of the vibration signal, rather than being excited by it incidentally (e.g. Pollack *et al.* 1998).

ALV probably conveys information primarily in the near-field sound modality. Whip spiders are nocturnal and ALV displays occur, and correlate with contest outcome, in complete darkness, which excludes visual detection (Fowler-Finn & Hebets 2006). Although vibrations might be transferred through the signaller's body to the substrate, whip spiders lack organs specialized for receiving such vibrations (Barth & Stagl 1976), indicating that they may be less sensitive to these than to air particle movements received by the trichobothria. Finally, physical contact between the vibrating antenniform leg and the receiver, as was previously thought to occur (Alexander 1962; Weygoldt 2000), was too infrequent to reliably convey display duration. Nevertheless, when such contact occurred, it was often with the leg regions bearing trichobothria, so these hairs could reliably detect contacts similarly to the long hair sensilla on some spiders' forelegs (Albert *et al.* 2001).

As discussed above, the form of ALV is suited to eliciting the maximum number of action potentials in the patella trichobothria over the total duration of the display. The evolutionary implications of this match are intriguing: since trichobothria have repeatedly been shown to function in predator or prey detection, and their response properties are well tuned to such a role, ALV may deliberately exploit these response properties, suggesting a sensory bias origin of signal form (e.g. Basolo 1990; Ryan 1990; Proctor 1991). The presence of trichobothria on leg regions typically stimulated during ALV may even suggest that they have been co-opted to function specifically in signal detection from an original predator detection role (e.g. Conner 1999).

The only previously proposed example of near-field sound communication via filiform hairs are the silent wing flicks of male African cave crickets that excite the filiform hairs of females (Heidelbach *et al.* 1991; Heidelbach & Dambach 1997). However, the exact nature of this signal is still unclear: wing flicks cannot be associated unequivocally with a receiver response; and it has not yet been shown that air movements, rather than visual or other stimuli, are the effective modality. In contrast, whip spider ALV duration clearly predicts contest outcome, and the trichobothria appear to be the only suitable means for its detection. This may also be the first clear demonstration of intraspecific airborne sound signalling of any kind by an arachnid: although courting spiders make audible sounds, it is substrate-borne vibrations that are sufficient to elicit a behavioral response in females (e.g. Barth & Rovner 1981). Spiders do have slit sensilla that could receive the air pressure component of these sounds (Barth 1982), and trichobothria that could receive air particle movements (e.g. Barth 2000), but experiments designed to test these airborne signal components could not isolate substrate-borne ones (Rovner 1967; Barth 1982). Regardless, considering the incredible sensitivity of arthropod filiform hairs, it would be surprising if near-field sound communication were not more widespread than these two examples (e.g. Barth 2000). The courtship behaviors of some whip spiders and true spiders would certainly seem probable candidates (Rovner 1980; Barth & Rovner 1981; Weygoldt 2000), but future work is needed to explore the evolutionary patterns and taxonomic breadth of various filiform hair functions and reveal the prevalence and importance of near-field communication.

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## ELECTRONIC SUPPLEMENTARY MATERIAL

Agonistic signals received by an arthropod filiform hair allude to the prevalence of near-field sound communication. Roger D. Santer & Eileen A. Hebets

## SUPPLEMENTARY METHODS

### (i) Analysis of behaviour

Whip spider contest behaviour was filmed at low and high-speed simultaneously. We used low speed films to characterise the contest behaviour of whip spiders in terms of contest phases observed, contest duration, and ALV display duration. We viewed films on a digital videocassette recorder (DSR-11, Sony Electronics Inc, USA) and standard television screen; the videocassette recorder's counter was used for time measurements.

We used high-speed films to analyse ALV in detail. We analysed 30 consecutive ALV cycles for each animal for the typical period and amplitude of antenniform leg vibration during a display. We used 1600ms filmed sections of ALV for each animal to observe the receiver body parts beneath a signaller's vibrating antenniform leg at 10ms intervals. Both analyses were carried out using Xcitex ProAnalyst Lite software (Xcitex Inc., Cambridge, MA, USA) on a standard PC.

### (ii) Stimulator design

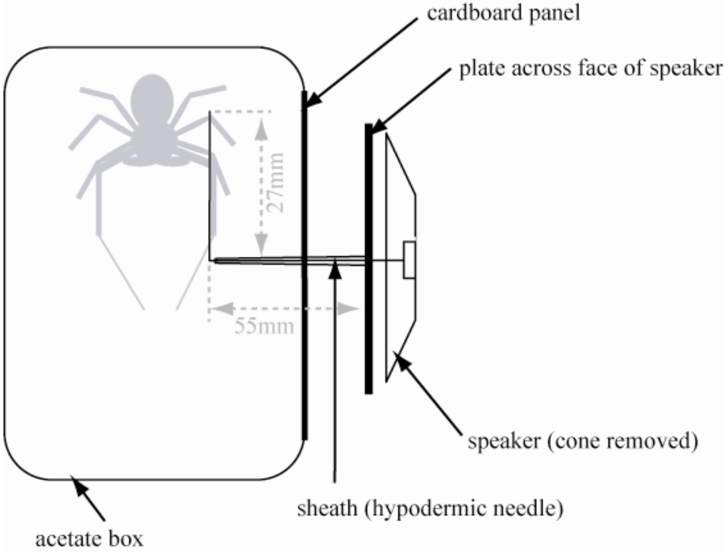
For practical reasons, we could not measure the air movements created by whip spider ALV displays directly. However, antenniform leg movements during ALV can be considered a dipole source and, as such, reproducing these movements with an object of the same size and shape will induce the same air movements.

Our stimulator moved a 27mm section of 200µm diameter tungsten wire (approximately the same diameter as a whip spider antenniform leg tarsus) from side to side in the horizontal plane with the same amplitude as real ALVs. These movements were controlled using the voice coil of a mini-speaker mounted on its side during experiments. Our set up during electrophysiological experiments is shown in fig S1.

We took several precautions to ensure that stimulation was only from movements of the tungsten wire and not the rest of the stimulator. (1) We cut away most of the speaker cone to reduce air movements; (2) we shielded the entire face of the speaker using a 2mm thick sheet of cardboard that overhung the edges of the speaker by 8mm; (3) the stimulus wire protruded through this sheet, perpendicular to the voice coil, but was sheathed by a no. 16 hypodermic needle for a length of 5.5cm from the speaker shield. At its exit from the sheath, the tungsten wire was bent at 90° to leave a 27mm horizontal section that served as the stimulus in our experiments (fig S1); (4) to exclude surface-borne vibrations, experiments were conducted on a vibration isolation table (TMC, Peabody, MA, USA). The stimulator itself was held in place by a clamp secured to the perimeter enclosure of the table, preventing any vibrations from affecting the preparation itself; (5) we further shielded our preparation by enclosing it in a box. This was a free-standing cardboard wall wider than our stimulator by 6.5cm. Attached to each side of this wall were sheets of clear acetate that we bent to completely encircle the preparation, leaving a small gap for electrode wires on the side furthest from the stimulator. On top of this encircling wall we placed an extra acetate sheet to create a box. The cardboard wall had a 5mm wide hole in its centre through which the hypodermic sheath of our stimulator protruded. The stimulator did not make contact with any part of this wall during experiments.

We carried out control experiments to confirm the effectiveness of this shielding (supplementary results).

**Fig S1:** Set up for simulating ALV displays. All parts of the stimulator, except the horizontally vibrating tungsten wire, were shielded from the preparation.



## SUPPLEMENTARY RESULTS

### (i) Typical contest behaviour

Although contests were filmed under full light conditions, contest behaviour was typical of that observed by previous authors (e.g. Fowler-Finn & Hebets 2006). Typical display behaviours were observed – pedipalp opening, antenniform leg vibration and pedipalp contact (Fowler-Finn & Hebets 2006); and contests escalated through characteristic, described phases (Weygoldt 2000; Fowler-Finn & Hebets 2006). Of 21 encounters staged, contests occurred in 19 (in 2, both competitors avoided one another without threat displays). Of these, 94.7% (18) involved an initial phase in which the opponents probed one another using their antenniform legs; 73.7% (14) escalated to a phase of ALV by one or both individuals; and 10.5% (2) escalated to full pedipalp contact. Mean contest duration, from initiation of a pedipalp threat display by one animal to retreat of the losing animal, was 191.6 ± 38.0s.

### (ii) Additional data on ALV positioning relative to the receiver

Figure 2a in our article shows the mean proportion of time during an ALV display that the signaller’s antenniform leg overlays particular receiver body regions. Table S2 lists the data used to produce figure 2a.

**Table S2:** The mean proportion of time during an ALV display (± SD) that the signaller's antenniform leg overlays particular receiver body regions. Proportions were calculated by recording antenniform leg position at 10ms intervals from 1600ms samples of ALV for each of 7 whip spiders and are illustrated in figure 2a of our article.

| Receiver body part below antenniform leg | Mean proportion of ALV duration | SD |
|---|---|---|
| **Body regions** | | |
| prosoma (cephalothorax) | 0.17 | 0.183 |
| opisthosoma (abdomen) | 0.22 | 0.183 |
| **Pedipalps** | | |
| left | 0.10 | 0.185 |
| right | 0.44 | 0.396 |
| **Antenniform legs** | | |
| left, proximal | 0.00 | 0.000 |
| left, distal | 0.00 | 0.000 |
| right, proximal | 0.56 | 0.419 |
| right, distal | 0.07 | 0.154 |
| **Walking legs** | | |
| left leg 2, proximal | 0.00 | 0.000 |
| left leg 2, distal | 0.00 | 0.000 |
| left leg 3, proximal | <0.01 | 0.002 |
| left leg 3, distal | 0.00 | 0.000 |
| left leg 4, proximal | 0.07 | 0.185 |
| left leg 4, distal | <0.01 | 0.002 |
| right leg 2, proximal | 0.71 | 0.360 |
| right leg 2, distal | 0.05 | 0.101 |
| right leg 3, proximal | 0.69 | 0.327 |
| right leg 3, distal | 0.15 | 0.238 |
| right leg 4, proximal | 0.29 | 0.169 |
| right leg 4, distal | 0.40 | 0.247 |

(iii) Electrophysiological control experiments

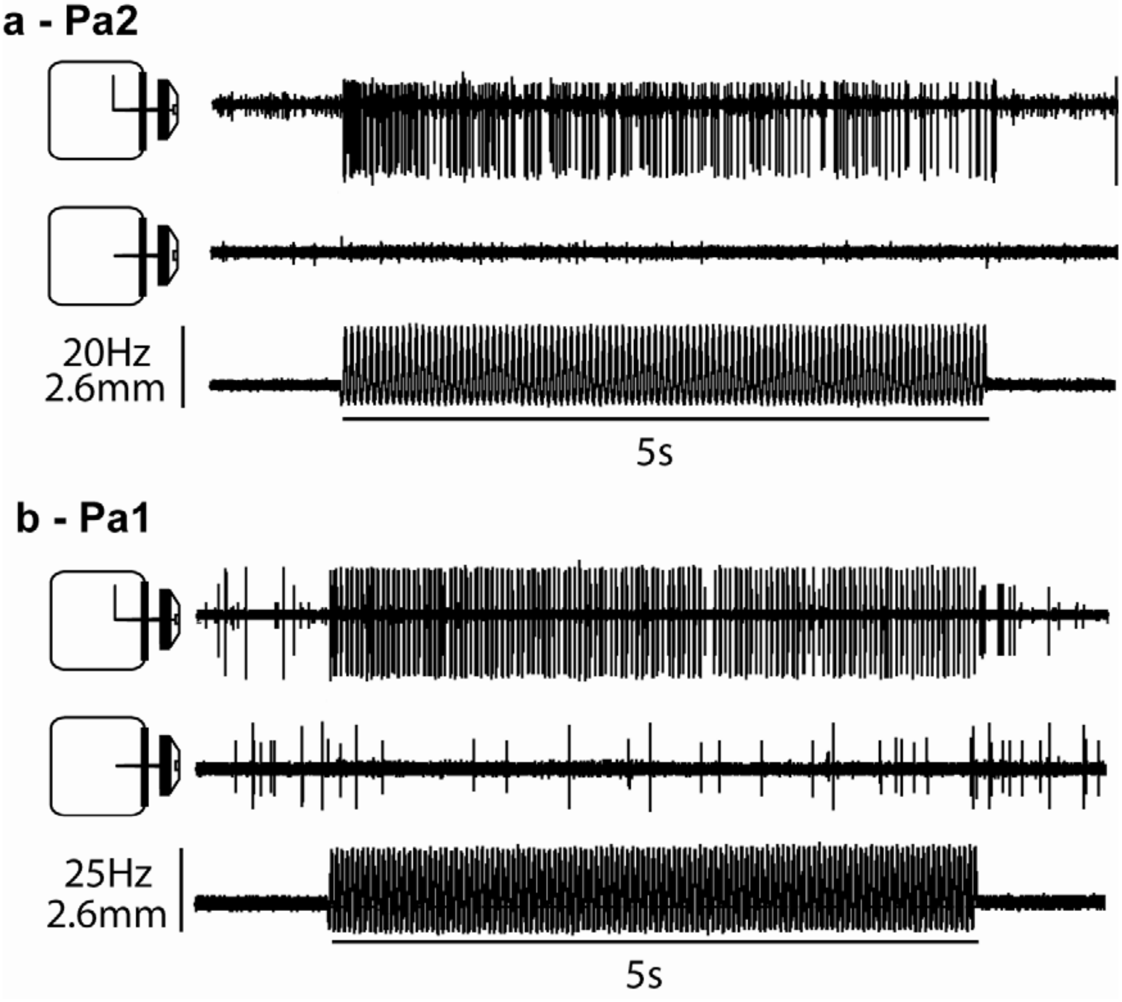
In order for air movements generated by our stimulator to reproduce those during real ALV, they must be generated only by side to side movements of the tungsten wire and not the remainder of our stimulator. We used shielding to ensure that this was the case (supplementary methods), and carried out control experiments to confirm this.

(1) We noted that moving the stimulus wire away from the trichobothrium caused a greatly reduced response. This was the case whether the stimulus wire was moved laterally away from the animal's body, or forwards, or vertically. In the latter cases, the speaker controlling wire movements was the same distance from the preparation as used during data collection. This is good evidence that air movement

stimulation comes from movements of the wire, rather than the speaker voice coil (data not shown).

(2) Cutting the tungsten wire at its 90° bend removed the horizontally-moving portion of wire that simulated ALV in our experiments. For trichobothrium Pa2, this stimulus configuration elicited no response when the stimulator was driven at any of the simulated ALV frequencies used in this study. For trichobothrium Pa1 (which showed a continuous level of background activity, even in the absence of stimulation), this stimulus configuration elicited no increase in activity from the background level (fig S3). Significantly more spikes were elicited by the stimulator with wire intact than were present as background activity during control experiments for both the large (paired t test: T=3.82, df=11, p=0.002) and small (T=3.57, df=11, p=0.004) action potentials across all stimulation frequencies. Since trichobothria are among the most sensitive air movement sensors known (Shimozawa et al. 2003), in both cases, stimulation is from horizontal movements of the tungsten wire and not the remainder of the stimulator and thus, the air movements generated should reproduce those elicited by real ALV.

**Fig S3:** If the horizontal tungsten wire portion is removed from the stimulator, the stimulator no longer excites trichobothrium Pa1 or 2.



(iv) Analysis of phase-locking

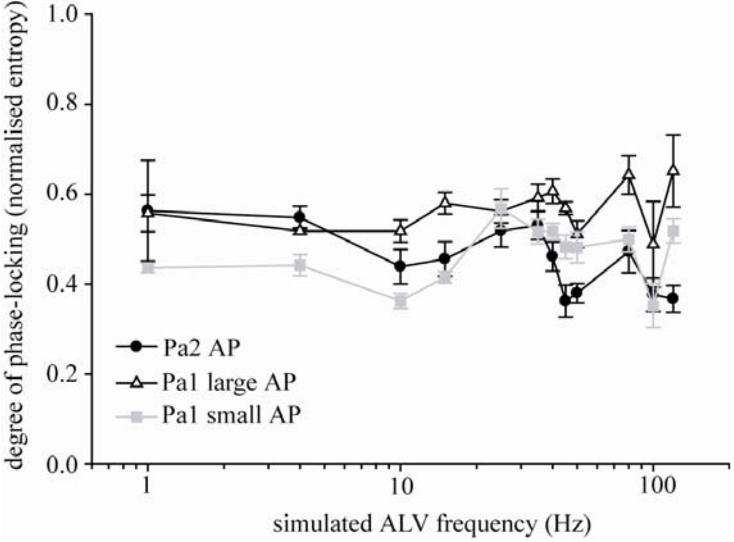
We measured the phase-locking of trichobothrium action potentials to simulated ALV using a normalised entropy index (Hurtado et al. 2004; Tort et al. 2007). For each trichobothrium response, we created phase histograms of action potential occurrences per 10° of phase bin (36 bins in total). We obtained an entropy measure (*h*) for these histograms (discarding those containing one action potential or less) by:

$$h = -\sum_{j=1}^{L} p_j \log p_j$$

where $L$ is the number of bins and $p_j$ is the probability corresponding to the $j^{th}$ bin. We normalised this entropy measure by the maximum entropy achieved for the uniform distribution ($h_{max}$): $p_j = 1/L$ for all $j$, and $h_{max} = \log L$. Therefore, normalized entropy, $h_N = (h_{max} - h)/h_{max}$. This varies between 0 and 1, with 0 indicating a uniform distribution and 1 indicating perfect phase locking in a single bin.

We calculated mean normalized entropy across 6 whip spiders for simulated ALVs of between 1 and 120Hz, for the Pa1 large action potential, Pa1 small action potential and Pa2 action potential (fig S4). In common with a previous study, we found this measure to be conservative, with values of around 0.4 still indicating strong phase-locking (Tort et al. 2007). Phase locking seemed to decline slightly with frequency for Pa2, but since high frequency stimuli elicited very few spikes it is difficult to assess the importance of this trend. Nevertheless, we tested whether normalized entropy varied with action potential type or simulated ALV frequency using a 2-way ANOVA. The ANOVA was significant (F=9.27, p<0.0001), but there was only an effect of action potential type (F=22.35, p<0.0001) and not simulated ALV frequency (F=0.92, p=0.34). There was no interaction between action potential type and simulated ALV frequency (F=0.42, p=0.65). Therefore, the degree of phase locking varies between the three types of action potential recorded, but is not affected by simulated ALV frequency over the range tested.

**Fig S4:** The phase-locking of action potentials from trichobothria Pa1 and 2 with simulated ALV frequency. Plot shows mean normalised entropy ± SEM.



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