© 2018. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2018) 221, jeb186247. doi:10.1242/jeb.186247

RESEARCH ARTICLE

Adult–larval vibrational communication in paper wasps: the role of abdominal wagging in *Polistes dominula*

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ABSTRACT

Communication through vibrational signals is widespread among social insects and regulates crucial social activities. Females of the social wasp *Polistes dominula* produce substrate-borne vibrations on the combs by performing a conspicuous abdominal oscillatory behavior, known as abdominal wagging. Several studies have reported correlative evidence in support of its signaling role, but direct evidence is still lacking. Because abdominal wagging is strictly associated with the presence of larvae in the nest and with cell inspection, it has been suggested that it could be involved in adult–larvae communication. According to this hypothesis, abdominal wagging vibrations would have short-term effects related to food and trophallactic exchanges between adults and larvae by modulating salivary secretion (decreasing its amount, to prepare larvae to receive food, or stimulating the release of larval saliva to adults). Here, by using an electro-magnetic shaker, we assessed, for the first time, the short-term effects of abdominal wagging on larval behavior by recording larval responses and by measuring the amount of saliva released immediately after abdominal wagging playback. Our results show that larvae are able to perceive the substrate-borne vibrations produced by abdominal wagging and react by increasing the movement of their body, possibly in order to attract the attention of adult females during feeding nest inspection. Yet, we found that vibrations neither increase nor decrease the release of larval saliva. Our results support the hypothesis of the alleged role of vibrations in adult–larvae communications; however, they do not support the long-lasting hypothesis of salivary release modulation.

KEY WORDS: Substrate-borne vibrations, Adult–brood communication, Playback experiment, Larval saliva, Larval behavior, Biotremology

INTRODUCTION

Substrate-borne vibrations represent a very ancient way to communicate in the animal kingdom (Hill, 2008). Despite their importance having been hypothesized since the beginning of the twentieth century, only the technological advancements of the last decades have facilitated the discovery of the widespread but relatively unexplored use of vibrational signals in many animal groups, and especially in complex insect societies (Cocroft and Rodríguez, 2005; Hunt and Richard, 2013; Cocroft et al., 2014). Phenomena such as honeybee pre-swarming vibrations, hunger signals made by mandible scratching in hornet larvae and alarm signals in termites performed by drumming their heads against the substratum clearly show that mechanical signals are widespread and regulate crucial aspects of insect societies (Ishay et al., 1974; Kirchner et al., 1994; Hölldobler, 1999; Visscher et al., 1999; Lewis and Schneider, 2000; Hunt and Richard, 2013). While many studies have found that several social insect species produce and use substrate-borne vibrations to communicate, we still know remarkably little about the function of many of these putative vibrational signals (Hunt and Richard, 2013). Although the use of airborne sound playbacks has a long history in ethological studies of diverse taxa (McGregor, 1992), substrate-borne vibrational playbacks have been conducted on a limited number of species of social insects (Kirchner, 1993; Hölldobler et al., 1994; Evans et al., 2007).

The playback of vibrations previously recorded on a substrate allows researchers to switch from correlational evidence to a direct test of signal function of the recorded vibrations, and thus to assess the identity of the receivers as well as the function of the signal. Indeed, the first steps toward this direction recently started to transform our understanding of intracolonial communication in insect societies (Evans et al., 2007; Suryanarayanan et al., 2011a,b; Hager and Kirchner, 2014; Jandt et al., 2017).

Paper wasps (Hymenoptera: Vespidae; Polistinae) represent a group of social insects where the use of substrate-borne vibrations is widespread (Brennan, 2007; Jeanne, 2009). The social life of these wasps occurs in nests built of paper material obtained by mixing wood fibers collected in the environment with saliva. Such paper nests facilitate the propagation of vibrations (Nascimento et al., 2005; Brennan, 2007), and indeed conspicuous oscillatory behaviors, a potential source of vibrations, have been described among independent-founding Polistinae with un-enveloped nests, i.e. the genera *Mishocyttarus*, *Belonogaster*, *Ropalidia* and in at least 18 species of the genus *Polistes* (Brennan, 2007; Jeanne, 2009). These behaviors consist of oscillatory movements of the entire body or its parts, i.e. antennae or abdomen, which in many cases hit the nest, probably producing substrate-borne vibrations. In many species of the genus *Polistes*, such as *P. fuscatus*, *P. metricus* and *P. canadensis*, body oscillations can be distinguished into three oscillatory behaviors: 'antennal drumming' (AD), 'lateral vibration' (LV) and 'abdominal wagging' (AbW) (Gamboa and Dew, 1981; West-Eberhard, 1986; Savoyard et al., 1998). AD consists of the wasp beating its antennae on the cell rims and is performed by females on the nest during food distribution to larvae (feeding context) (Pratte and Jeanne, 1984). LV and AbW are instead generally performed during adult–adult interactions and cell inspection (non-feeding context); LV consists of the wasp standing on the comb shaking its abdomen so vigorously that in

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Received 21 June 2018; Accepted 12 August 2018

Journal of Experimental Biology (2018) 221, jeb186247. doi:10.1242/jeb.186247

some cases the oscillation can involve the entire body and produces an audible sound (Gamboa and Dew, 1981; West-Eberhard, 1986; Savoyard et al., 1998); AbW consists of a longer and slower lateral vibration of the abdomen performed by the wasp while walking over the cells (Gamboa and Dew, 1981). Although two or all three kinds of behavior can be present in the same species (Gamboa and Dew, 1981; Harding and Gamboa, 1998), in one of the most studied species of the genus, *Polistes dominula*, only one type of body oscillation is known. This was called abdominal wagging but it shows intermediate temporal and behavioral features between the LV and AbW described for other species (such as *P. fuscatus* and *P. metricus*; Brennan, 2007; Jeanne, 2009). In *P. dominula*, AbW is performed not only in the non-feeding context but, similar to AD, also in the feeding context (Brillet et al., 1999). The AbW of *P. dominula* is one of the few oscillatory behaviors where the substrate-borne vibrations produced have been recorded and analyzed (Brennan, 2007). When a wasp performs AbW, it transmits substrate-borne vibrations into the paper nest. Such vibrations are characterized by the alternation of short gaps of silence and broadband pulses, which are generated by the contact between the wasp's abdomen and the nest surface. Most of the energy of the pulses is concentrated on low frequencies (Brennan, 2007).

As AbW is a very evident, frequent and vigorous behavior in *P. dominula* colonies, it has been suggested that AbW might be energy expensive (Jeanne, 2009) and it may represent a vibrational signal (Brillet et al., 1999; Brennan, 2007; Jeanne, 2009). Moreover, because AbW is strictly associated with the presence of larvae in the nest and is usually performed during nest inspection (Brillet et al., 1999; Brennan, 2007), a behavior that includes adult–larva contact and food exchange (Brennan, 2007), it has been suggested to represent an adult–larva communication signal (Jeanne, 2009). However, the function of AbW represents a long-standing and ongoing debate in wasp sociobiological studies (Gamboa and Dew, 1981; Gamboa et al., 1990; Savoyard et al., 1998; Brillet et al., 1999; Brennan, 2007; Ishikawa et al., 2011; Mignini and Lorenzi, 2015).

It has been proposed that AbW vibrations could have short-term effects related to food and trophallactic exchanges between larvae and adult females performing AbW. A specific hypothesis is that AbW could regulate the secretion of saliva from larvae to soliciting adults (salivary release modulation hypothesis). Larval saliva is indeed rich in sugars and amino acids, and represents an attractive source of nourishment for adult wasps (Hunt et al., 1982); therefore, it has been proposed that adult–larvae trophallaxis could have played a role in the establishment and maintenance of sociality in wasps (Hunt, 1991). As both AbW and salivary exchange from larvae to adults are frequent during cell inspections (Hunt at al., 1982; Hunt, 1991; Brillet et al., 1999; Brennan, 2007), AbW could represent a request for saliva from the adult to the larvae, in which case vibrations would stimulate the release of this nourishing secretion (modulation by stimulation) (Savoyard et al., 1998). An alternative proposal is that AbW instead modulates salivary secretion by decreasing its amount, in order to prepare larvae to receive food (modulation by inhibition) (Brennan, 2007; Jeanne, 2009). This would be supported by the observation that AbW is frequently associated with food distribution to larvae by adult females that return to the nest after foraging (Brillet et al., 1999; Brennan, 2007; Jeanne, 2009). Given that AbW is performed during solid food distributions, it seems unlikely that it could be linked to a modulation by stimulation, as adult females are indeed unable to imbibe larval saliva and simultaneously keep food between the mandibles (Pratte and Jeanne, 1984; Brillet et al., 1999; Jeanne, 2009). Moreover, in the American species *P. fuscatus*, a correlative study found a moderate decrease in larval saliva release after LV (Cummings et al., 1999).

Another (but not mutually exclusive) hypothesis proposes that AbW might have long-term effects on larval phenotype, eventually influencing caste fate. The idea is that vibrations would interfere with the developmental pathway of larvae, which would be biased toward the worker phenotype (Jeanne, 2009; Mignini and Lorenzi, 2015). The vibration effect on the phenotype has been recently demonstrated for *P. fuscatus*, in which AD on cell rims produces vibrations that interfere with the food pathway to bias larval fate toward a worker phenotype (Suryanarayanan et al., 2011a; Jandt et al., 2017).

The evidence gathered so far about the possible AbW function is thus contrasting and mainly correlative. To our knowledge, no direct tests of abdominal wagging function have been performed. Here, we therefore tested for the first time the short-term effect of AbW on larval behavior in *P. dominula*. We recorded and played back AbW vibrations on the nest by using an electro-magnetic shaker, and tested (a) whether larval motion behavior changes immediately after AbW vibrations, in order to assess whether larvae are able to perceive the vibration stimulus produced by adult behavior in the absence of other stimuli (i.e. visual or chemical ones); and (b) whether larval salivary release changes (increase or decrease) immediately after AbW vibrations, as expected by the salivary release modulation hypothesis. Larvae of different developmental stages were tested, as larval responsiveness to vibrational stimuli might change according to larval developmental stage (Brillet et al., 1999; Suryanarayanan et al., 2011a,b). Moreover, as AbW is performed mainly in the pre-emergence period and its frequency drastically decreases at the emergence of first workers (Brillet et al., 1999), we carried out the playback experiments in two different periods of the colonial cycle to evaluate whether larval response differs between the pre- and post-emergence phases. Our experimental design allowed us: (i) to evaluate the effect of AbW on larvae in the absence of other potential signals; (ii) to test the previously hypothesized short-term effect of AbW; and (iii) to assess the responsiveness of larvae and how this varies during individual and colony life.

MATERIALS AND METHODS

Studied species

Polistes dominula (Christ 1791) is a widespread European primitively eusocial wasp. Colonies are founded in early spring when one or more females build a new colony. At the end of May, the first emerging females are workers that take care of the nest and the immature brood and do not reproduce. Reproductive individuals, males and gynes, emerge only later in the season, from the end of July (Reeve, 1991). Mating occurs outside of the colony at the end of summer (Beani, 1996); mated females overwinter in large groups and then start new colonies the following spring (Dapporto and Palagi, 2006).

Animal collection and laboratory rearing

Polistes dominula colonies (n=60) were collected around Florence, Italy, in two distinct stages of the colonial cycle: 33 of them in pre-emergence phase, at the beginning of May, and 27 in post-emergence phase (before the start of emergence of reproductive individuals) at the beginning of July. All colonies were transferred to the laboratory where they were reared in glass cages (15×15×15 cm) with water, sugar and fly maggots *ad libitum*. All cages were kept under natural temperature and light conditions with

additional illumination from neon lighting. At the beginning of the experiment, nest size (number of cells) was 42.4±15.6 (mean±s.d.) cells for pre-emergence colonies and 77.5±48.2 cells for post-emergence colonies, and there were 2.2±1.3 females on pre-emergence colonies and 11.7±7.5 females on post-emergence ones. All colonies had brood of various stages (i.e. pupae, larvae and eggs). Colonies allocated to different treatments (AbW, WN, control; see below) did not differ for any of the investigated parameters, in either the pre-emergence group or the post-emergence group (Kruskal–Wallis test, $P>0.05$ in all cases; see Table S1). Larvae used in the experiment were classified into two size classes: medium (corresponding to 3rd and 4th instar) and large (corresponding to 5th instar). First and second instar larvae were not included in this study as it was not possible to collect saliva or to properly record behavior given their small size. To avoid any bias due to different phenotypes of larvae sampled in the two sets of experimental colonies (i.e. worker larvae in the pre-emergence period and reproductive larvae in the post-emergence period), we performed experiments only on larvae destined to become workers. In the colony cycle of a typical temperate *Polistes* species, the reproductive phase starts with the emergence of a first cohort of males, followed by reproductive females (proterandry) (Bulmer, 1983; Cappa et al., 2013). We thus checked our colonies for 2 weeks after the experiment in order to confirm that all newly emerged individuals (corresponding to the tested larvae) were females (and thus workers, having emerged before the first cohort of males; Cappa et al., 2013). Finally, rearing conditions were standardized to ensure that any differences between pre- and post-emergence phase larval behavior and in the amount of saliva released were not due to differences in food and liquid provisioning.

Playback trials

Colonies ($n=60$) were randomly allocated to one of three groups that received a different playback stimulus: AbW, the biologically meaningful vibrational track and two control tracks: white noise (WN), a random signal having equal intensity at different frequencies that controls for the effect of non-specific vibrations; and a silent control track to assess possible effects of the experimental apparatus. Both AbW and WN were artificially made by means of Adobe Audition 3.0 (Adobe Systems Inc.). The specific temporal and spectral features of AbW were chosen taking into account both published data (Brennan, 2007) and our own recording inventory (see Table S2). AbW was 2 min long with a rate of 2.5 events per minute (i.e. AbW events were regularly spaced 26 s from each other). The WN track was artificially created by replacing AbW events with WN events of identical intensity and duration (see Fig. S1). In particular, WN had the following features: (1) the total duration of the track was the same as that of the AbW track; (2) the track contained a repetition of noise elements that had the same duration as the AbW elements and were separated from each other by the same interval of silence; (3) the frequency range was the same as that of the AbW (frequency range 0–5000 Hz); (4) unlike the AbW track, the energy was equally distributed throughout the frequency range; (5) it did not have the pulsed structure typical of AbW; thus, the energy was equally distributed along the duration of each element; (6) the intensity of WN (minimum 28.1 µm s^{-1}, maximum 549 µm s^{-1}) was comparable with the intensity of the AbW track (minimum 31.5 µm s^{-1}, maximum 422 µm s^{-1}). This allowed us to control for the non-specific effect of vibrational noise of equal intensity, while preserving the spectral and temporal features of the biologically relevant stimulus, AbW. Examples of the two tracks are given in Audio 1 (AbW) and Audio 2 (WN).

Playbacks were transmitted by an electro-magnetic shaker (CBC Europe, Milan, Italy) that was fastened to a plastic wire (diameter 0.4 cm) using a U-shaped metal screw that was inserted into the shaker's tip (see Fig. S2 for the experimental apparatus). Nests were attached with a metal string to the wire; a small quantity of wax was used to ensure the nest–wire connection. The shaker was positioned 20.5±0.1 cm from the nests.

A laser vibrometer (Ometron VQ-500-D-V, Brüel and Kjær Sound and Vibration A/S, Nærum, Denmark) was used to ensure that the AbW and WN playbacks were correctly transmitted to the nests and the first one resembled the naturally occurring AbW produced by a female wasp (Table S2). Recordings were digitized with a 48 kHz sample rate and 16-bit depth, and stored directly onto a hard drive through a multichannel LAN-XI data acquisition device (Brüel and Kjær Sound and Vibration A/S). Spectral features were analyzed with Pulse 14.0 (Brüel and Kjær Sound and Vibration A/S) after applying fast Fourier transform (FFT) with a window length of 400 samples and 66.7% overlap and a Blackman–Harris window.

Twenty-four hours before the playback experiment, protein food was removed from the rearing cages of colonies, in order to standardize as much as possible the protein nutritional status of larvae of different colonies. All adult wasps were removed from colonies before the playback experiments.

Behavioral data collection

Larval movements, such as body contraction, head erection and pecking, have been widely used in social insects as a proxy for begging signals [see examples for several taxa of social insects in Hunt, 1988 (wasps); Creemers et al., 2003; Kaptein et al., 2005 (ants); Kawatsu, 2013 (termites)]. The behavioral repertoire of wasp larvae, reared in narrow cells, is restricted to a few behavioral patterns, mainly related to interaction with adult nest inhabitants. In *Polistes*, no information exists about potential larval signals (such as hunger signals); we thus decided to use body and palpi movements as indices of larval response (see below for details). These behaviors represent the only measurable reactions that a larva, deeply inserted into a cell, can show. Moreover, a preliminary experiment confirmed that such behaviors are related to feeding context, thus representing a potentially adaptive response to signals from adults. First, larvae that moved at least once were more likely to receive a delivery of food than larvae that did not move (Fig. S3). Moreover, the number of body and palpi movements were correlated with the number of food visits by adult wasps (food visits versus body movements: number of events, Spearman $\rho=0.361$, $P<0.001$; food visits versus palpi movements: number of events, Spearman $\rho=0.304$, $P<0.001$, $n=102$ larvae from 13 colonies, each observed for 15 min during the feeding context).

The behavioral response of larvae during the 2 min of playbacks was recorded through a video camera (HDD DCR-SR36, Sony Corporation Minato, Tokyo, Japan) directed at the cell's opening on the comb. We used the software BORIS 4.1.1 (Department of Life Science and System Biology, University of Torino, Italy; www.boris.unito.it; Friard and Gamba, 2016) to annotate larval behavior. For each larva, we recorded the number of movements it performed, considering the two classes of movement: (i) movement of the entire body, i.e. lateral and/or up–down movement of the body, excluding movement of the palpi; (ii) palpi movement, i.e. opening/closing of the mouth by moving the palpi (see Movie 1). Video recordings were annotated without knowing which treatment they belonged to.

Only those larvae that were completely visible from the video recordings, i.e. fully enabling the assessment of body and palpi movements, were retained for the analysis. Overall, 203 larvae from 54 colonies were tested (mean±s.d. number of larvae tested per nest=3.8±1.3; sample sizes for each category are given in Table S3).

To determine whether the baseline behaviors of larvae (body movements and palpi movements) were different among colonies allocated to different treatments (AbW, WN and control), we also video recorded colonies 1 min prior to treatments and analyzed behaviors in the same manner.

Saliva collection

Under natural conditions, after a bout of AbW, a foundress can sequentially inspect one or more larvae (Brillet et al., 1999; up to eight larvae, I.P., A.C., personal observation). Cell inspection and solicitation of larval saliva can last for a variable amount of time (from rapid cell inspections of less than 1 s to longer ones of up to 30 s; I.P., A.C., personal observations) and the first larva to be inspected by the foundress after the AbW is not usually the closest one to the wagging individual (Brennan, 2007). This implies that within a colony some larvae may be solicited soon after the AbW bout, while others may receive cell inspection and solicitation well after. To evaluate the effect of vibrations on larval saliva release, we developed a stimulation protocol that mimicked this phenomenon, by modifying the saliva collection protocols in Pratte and Jeanne (1984), Cummings et al. (1999) and Turillazzi et al. (2004).

Saliva was collected immediately after the end of the 2 min of playback: briefly, we gently stimulated the mouthparts of each larva with a 5 µl calibrated transparent glass microcapillary (Blaubrand®) and recorded the volume of saliva released after 1 min of stimulation. Up to six larvae per nest were sequentially stimulated and their saliva collected. A pilot experiment showed that the order of larval solicitation did not have an effect on the amount of saliva released (Pearson correlation, ρ=0.003, P=0.972, n=194).

Preliminary analyses were carried out to infer the appropriate time interval of stimulation to sample saliva. Fifteen larvae from two nests were stimulated on the mouth with a 5 µl calibrated transparent glass capillary tube and the amount of saliva released was recorded every 30 s for 3 min. The amount of saliva released rapidly dropped as time passed, with the largest fraction of the total amount released by larvae within the first 60 s (90.4±10.8%, mean±s.e.m., of the total amount; Fig. S4). In the first 30 s, the amount of saliva released was smaller (on average, 79.0±18.0%, mean±s.e.m.) but the coefficient of variation was greater (22.8 compared with 11.9 for the 60 s interval). So, we performed analysis at both 30 and 60 s to increase the likelihood of detecting any effect of the treatment. As the results were largely overlapping, we report only the results from the 30 s interval (those related to the 60 s interval can be found in Table S5). Overall, 282 larvae from 59 colonies were tested (mean ±s.d. number of larvae tested per nest=4.70±1.47; sample sizes for each category are given in Table S3).

Statistical analyses

Non-parametric one-way ANOVA (Kruskal–Wallis test) was used to assess differences in terms of the number of cells, number of adult wasps and number of brood among colonies allocated to the three treatments (AbW, WN, control), as data were non-normally distributed. A χ^2 test was used to verify that baseline larval behavior (number of body and palpi movements) was not different among colonies allocated to the three treatments (AbW, WN, control) before the playback experiment. To assess the influence of treatment, colony stage (pre- and post-emergence) and larval size (medium and large) on larval behavior, while also accounting for the non-independence of data (i.e. larvae belonging to the same colony), we used a generalized estimating equation (GEE) approach, which extends the generalized linear model to allow analysis of correlated observations such as clustered data. This approach is robust against misspecification of the error structure model and it is more relaxed on distributional assumptions (Burton et al., 1998; Overall and Tonidandel, 2004; Hubbard et al., 2010). For all GEEs, model selection was performed on the basis of the 'quasilikelihood under the independence model' criterion (QIC) by choosing the model parameters that resulted in the smallest QIC (Pan, 2001). We used two GEE with the following parameters: Poisson distribution with log function, independent working matrix and type III sums of squares. Dependent variables of each GEE were, respectively, (a) the number of times a larva was seen moving its body and (b) the number of times a larva was seen moving its palpi. For both GEE, treatment, colony stage and larval size were set as categorical explanatory (fixed) factors, and colony as random explanatory factor (as only one observation was done on each larva, but many larvae were recorded from the same colony). As many larvae did not show any movement, we also ran a binary model, i.e. considering as positive those cases in which larvae moved at least once, and as negative those in which larvae never moved, and using GEE with a binary logistic distribution. As the results are largely overlapping, we report only the results related to the number of movements (results for binarized data can be found in Table S4).

To assess the influence of treatment, colony stage and larval size on saliva release, we used GEE, with a normal probability distribution, an identity-link function and an independent working correlation matrix. We log-transformed the amount of saliva released to obtain a gaussian distribution (log-transformed distribution: Lilliefors-corrected Kolmogorov–Smirnov test, D=0.047, d.f.=282, P>0.200). The amount of saliva released (log-transformed) by each larva was the dependent variable, while treatment (AbW, WN, control), colony stage (pre-emergence versus post-emergence) and larval size (large or medium) were the fixed categorical explanatory factors and colony was the random explanatory factor (as only one observation was done on each larva, but many larvae where recorded from each colony). To calculate the effect size, we computed Cohen's d as: $d=(m_a-m_b)/\text{s.d.}$ where m_a and m_b are the estimated marginal means of each category within the pairwise comparison, and s.d. is the pooled standard deviation. According to Cohen (1969), the interpretation of d is as follows: d=0.2: small effect, d=0.5: medium effect, d=0.8: large effect. For all GEE models, *post hoc* comparisons using Bonferroni sequential correction were performed. Statistical analyses were performed in SPSS 20.0 (SPSS, 2011).

RESULTS

Larval movement experiment

Before stimulation with the playback, larvae were almost motionless and no difference in the likelihood of moving was observed in larvae on nests allocated to different treatments (body movements: average 9.13%, χ^2=3.82, d.f.=2, P=0.148; palpi movements: average 5.20%, χ^2=0.813, d.f.=2, P=0.666).

When subjected to the vibrational playback experiment, the number of body movements performed by larvae was influenced by vibrational treatment, larval size and colony phase (GEE, Table 1, Fig. 1). Larvae subjected to AbW (n=70) moved significantly more than both those subjected to WN (n=66; 2.03 times more, AbW versus WN: P=0.010, effect size d=0.46) and those in the control treatment (n=67; 9.90 times more; P=0.007, effect size d=0.95) (Fig. 1A).

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Table 1. Influence of treatment, colony phase and larval size on the number of larval body movements

Source	Type III Wald χ^2	d.f.	*P*-value
(Intercept)	0.456	1.000	0.500
Treatment	**40.027**	**2.000**	**<0.001**
Colony phase	**7.650**	**1.000**	**0.006**
Larval size	**4.028**	**1.000**	**0.045**
Colony phase×treatment	0.960	2.000	0.619
Treatment×larval size	1.630	2.000	0.443
Colony phase×larval size	1.140	1.000	0.286

Results from generalized estimating equation. Significant explanatory variables are in bold.

Larvae subjected to WN moved 4.87 times more than those in the control treatment (P<0.001, effect size d=0.67) (Fig. 1A).

Large larvae (n=125) were 1.70 times more likely to move at least once than medium-sized ones (n=78) (Fig. 1B; P<0.037, effect size d=0.26). Finally, pre-emergence colony larvae (n=103) were 2.25 times more likely to move at least once than post-emergence colony larvae (n=100) (Fig. 1C; P=0.013, effect size d=0.35). No effect of an interaction among any of the factors was found.

In contrast, when considering as a variable the number of times a larva moved its palpi, no significant effect was detected for any factor or for any interaction of factors, except for a significant effect of larval size (GEE, Table 2, Fig. 1; large larvae moved their palpi about 2.78 times more than medium larvae, P=0.022, effect size d=0.36). Frequency distributions for the behavioral variable values according to factors and their interactions are reported in Fig. S5.

Saliva release experiment

Different treatments (AbW, WN, control) had no effect on the amount of saliva released by stimulated larvae, while both colony stage and larval size had a significant effect (GEE, Table 3, Fig. 2): larvae from pre-emergence colonies released on average 1.38 times more saliva than larvae from post-emergence colonies (P<0.001, effect size d=0.46, n=123 versus 159 larvae from pre- and post-emergence colonies, respectively; Fig. 2C); large larvae released on average 1.21 times more saliva than medium larvae (P=0.003, effect size d=0.35, n=182 large versus 100 medium larvae; Fig. 2B). Frequency distributions for the amount of saliva released according to factors and their interactions are reported in Fig. S5.

DISCUSSION

Experiments conducted to stimulate *P. dominula* larvae with substrate-borne vibrations played back onto paper nests showed that *P. dominula* larvae can perceive vibrations produced by AbW and react by increasing their body movement. The behavioral changes of larvae after AbW playback indicate, for the first time, the role of the AbW in adult–larva communication in *P. dominula*, highlighting its vibrational nature. Indeed, playing back on the nest surface a previously recorded putative vibrational signal without the physical presence of the signaler allowed us to exclude that larvae were responding to any visual signal or to pheromone released from abdominal glands in association with abdominal wagging. Chemical communication is predominant in social insect societies and the spread of olfactory signals is often accompanied by a variety of specific behaviors and movements that favor the emission and diffusion of chemicals (Vander Meer and Alonso, 1998; Vander Meer et al., 1998). Playback experiments that rebroadcast natural vibrational signals on the colonies and observe the response of colony members give us the opportunity to discriminate among behavioral patterns linked to different communication channels and so to deepen our knowledge of the multiple communication modalities used in social insect colonies (e.g. Röhrig et al., 1999; Casacci et al., 2013).

Moreover, to our knowledge, this is the first direct evidence of a larval response to an adult delivered signal and thus the first experimental demonstration of communication from adults to larvae in *Polistes* wasps. Among social insects, larvae of many different species are either reported or hypothesized to produce mechanical or chemical signals to inform adults of their needs, i.e. hunger signals. Pioneering investigations demonstrated that hornet larvae may communicate via substrate vibrations, by showing that *Vespa orientalis* larvae perceive vibrations and respond to them by emitting their own substrate-borne vibrations, which can be produced by body contractions and by scratching on the paper nest (Ishay and Ikan, 1968; Ishay and Landau, 1972; Ishay and Brown, 1975; Barenholz-Paniry and Ishay, 1988; Barenholz-Paniry et al., 1988). Subsequently, hunger signals of varying nature have also been demonstrated for ants (Cassill and Tschinkel, 1995; Kaptein et al., 2005), bumble bees (Pereboom et al., 2003) and honeybees (Huang and Otis, 1991; Heimken et al., 2009). The opposite situation, however, that adults produce signals to communicate with larvae, has been poorly investigated and our results open a new intriguing research avenue.

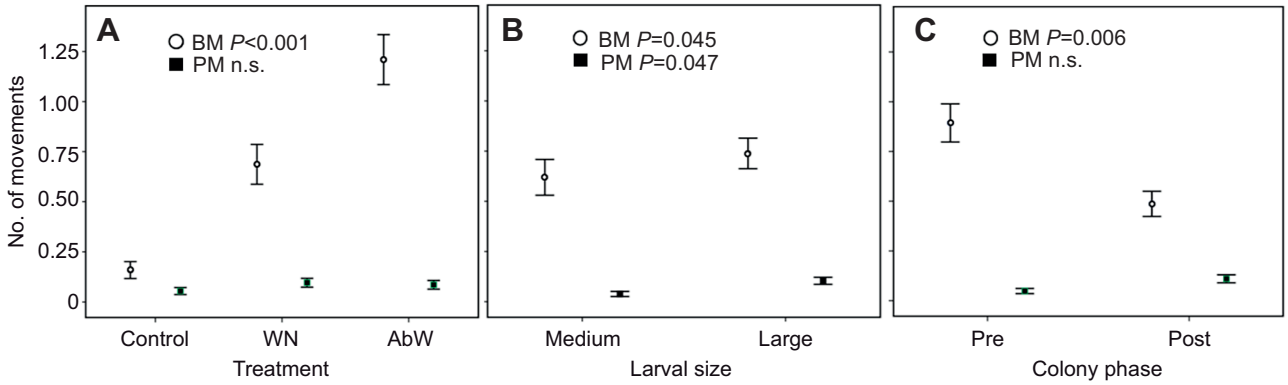

Fig. 1. Larval body and palpi movements. Effect of (A) treatment (WN, white noise; AbW, abdominal wagging), (B) larval size and (C) colony phase (pre- versus post-emergence) on the body and palpi movements of larvae. Means±s.e.m. are reported for the number of body movements (BM) and the number of palpi movements (PM). n.s., no significant difference.

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Table 2. Influence of treatment, colony phase and larval size on the number of larval palpi movements

	Type III		
Source	Wald χ^2	d.f.	*P*-value
(Intercept)	**83.217**	**1.000**	**<0.001**
Treatment	0.088	2.000	0.957
Colony phase	1.814	1.000	0.178
Larval size	**3.959**	**1.000**	**0.047**
Colony phase×treatment	0.606	2.000	0.739
Treatment×larval size	1.160	2.000	0.560
Colony phase×larval size	0.030	1.000	0.862

Results from generalized estimating equation. Significant explanatory variables are in bold.

An outstanding question is: what is the function of such adult signals? Our experimental approach does not support the salivary release modulation hypothesis proposed for this behavior (Savoyard et al., 1998; Brennan, 2007; Jeanne, 2009). Although our stimulation protocol on larval mouthparts only partially replicates the natural stimulation performed by adult wasps in soliciting larval saliva emission, we are confident that our method did not stress larvae as we did not observe any difference from what normally occurs after an AbW bout, i.e. sequential inspection of several cells and solicitation through adult–larva mouth contact. So, we expected that if AbW played a role in modulating the release of saliva, the experimental larvae that experienced the vibrations produced by AbW (track) would change the amount of released saliva with respect to control larvae. Our data show that the AbW treatment did not affect the amount of saliva released with respect to control treatments (control and WN), therefore suggesting that AbW vibrations have no effect on the regulation of larval saliva release, as stated by Brennan (2007) and partially supported by correlative evidence in *P. fuscatus* (Savoyard et al., 1998; Cummings et al., 1999). The finding that no difference existed between the no-vibration control and the WN control (where non-specific vibrations were given) suggests not only that the vibrations produced by AbW did not have that function but also that, in general, non-specific vibrations themselves do not influence salivary release. Salivary release was instead influenced by two factors: larval size and colony stage. As expected, large larvae released more saliva than medium ones. This can be easily explained as an effect of the overall size, and thus the 'crop' size of large larvae, i.e. we might suppose that the amount of saliva stored in a larva is a function of its crop size, which would change with overall size (Suryanarayanan and Jeanne, 2008). Regarding colony stage, larvae from pre-emergence colonies released more saliva than larvae from post-emergence colonies, independently from larval size.

Table 3. Influence of treatment, colony phase and larval size on the amount of larval saliva released in 30 s

	Type III		
Source	Wald χ^2	d.f.	*P*-value
(Intercept)	**538.770**	**1**	**<0.001**
Treatment	0.004	2	0.998
Colony phase	**15.927**	**1**	**<0.001**
Larval size	**8.866**	**1**	**0.003**
Treatment×colony phase	0.071	2	0.965
Colony phase×larval size	2.314	1	0.128
Treatment×larval size	3.030	2	0.220

Results from generalized estimating equation. Significant explanatory variables are in bold.

An intriguing potential explanation is that the effect could be linked to the receivers of saliva. In pre-emergence colonies, the only saliva receivers are the queen (larvae's mother) and the subordinate foundresses (which can inherit the colony later; Queller et al., 2000; Leadbeater et al., 2011), while in post-emergence colonies, the most likely receivers are workers, i.e. non-reproductive individuals. As saliva production is an energetically costly task for a larva, natural selection would favor a modulation of saliva release from larvae according to the benefits/costs tradeoff linked to the colony's nutritional status and identity of the receivers. By offering saliva to foundresses during times of low nourishment, such as the early phase of the nesting cycle, larvae could strongly promote the survival and the reproductive output of reproductive individuals, i.e. foundresses (West-Eberhard, 1969; Hunt, 2007). Later in the nesting cycles, such as in post-emergence colonies, larvae are tended by alloparental offspring of the colony. These live in a high-nourishment environment and will rarely become reproductive, and are thus more expendable (workers) (Hunt, 2007). This resulting pattern, i.e. less-well nourished larvae early in the colony cycle passing more saliva than better-fed larvae later in the colony cycle, has been hypothesized by Hunt (2007) and is strongly supported by our data.

In contrast, the behavioral results of our playback experiments show that general body movement was not the same when larvae were stimulated with the AbW track or with controls. First, in general, vibrations (both WN and AbW) provoked a higher degree of body movement (lateral and vertical) by larvae than the silent control. More interestingly, however, the specific vibrations produced by AbW elicited more movements (roughly double) than those elicited by the non-specific white noise track. As AbW and WN did not differ in their vibrational intensity, this suggests that the response of the larvae is not a simple and non-specific reaction to vibrational noise, but rather that the specific vibrations of AbW (i.e. the temporal and/or the spectral pattern: AbW consists of a sequence of discrete pulses with the energy peak at the dominant frequency, whereas WN is a continuous sinewave with constant energy throughout the spectrum) are perceived and evoke a behavioral response in larvae that is greater than the response evoked by non-specific vibrations. This suggests the need for further investigations to disentangle the relative importance of spectral and temporal parameters of AbW in the degree of larval behavioral response. It is known that even apparently small differences in vibrational signal parameters (i.e. frequency span, intensity) can produce significant differences in terms of behavioral responses of stimulated individuals in insects (e.g. De Groot et al., 2011; Suryanarayanan et al., 2011b; Polajnar et al., 2014; Mazzoni et al., 2015). While we currently have no unambiguous experimental evidence regarding the actual function of larval movement, as body movement may be energetically costly for larvae, we can speculate on the possibility that larval movements represent response signals to the adults' call, in order to attract the attention of adult females during feeding and nest inspection, with which AbW is often associated (Brillet et al., 1999; Brennan, 2007). Indeed, a relationship between larval body movements and adult inspection has been found in *Mischocyttarus* paper wasps (Hunt, 1988). The results of our preliminary experiment (reported in Fig. S3) show a clear association between larval body movement and feeding context by supporting that larval body movement is not a non-specific response to vibrational noise but could represent an adaptive response that, potentially, increases the likelihood of food delivery from adults to larvae. Although we recognize that the function of the larval response to AbW needs a more robust demonstration, overall,

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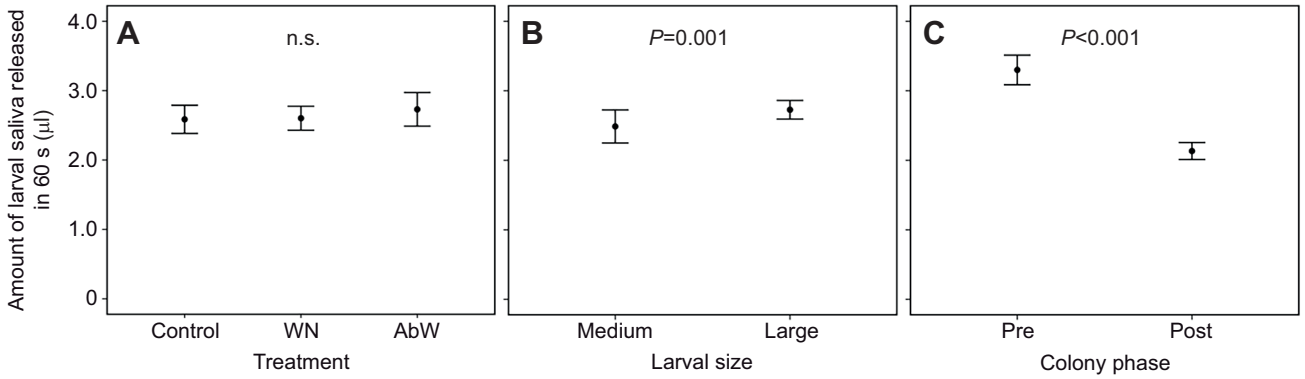

Fig. 2. Release of saliva. Effect of (A) treatment, (B) larval size and (C) colony phase on the amount of saliva released by larvae in 60 s. Means±s.e.m. are reported.

our results show that larvae perceive vibrations and thus suggest that AbW should be interpreted as a specific vibrational behavior produced by adult females and perceived by larvae, which respond to this in a measurable way.

Like the release of saliva, larval movement was also influenced by larval size and colony stage. Large larvae moved more than medium-sized ones. This can be explained by considering the more advanced developmental stage of large larvae, which might need a higher degree of care (e.g. food) by adults and/or might be more able to move. Larvae from pre-emergence colonies moved more than larvae from post-emergence colonies. This could be explained by the necessity of larvae in pre-emergence combs to be able to attract the few adults present on the colony. After worker emergence, this urgency to attract adults is probably decreased by the contemporaneous presence of many individuals on the nest. The lack of difference in palpi movements (a behavior linked to feeding) among treatments could be due to the absence of adults on the nest. Perhaps to reduce energy costs, each larva moves the palpi only at the moment in which a female, after returning to the nest with food, inserts her head into the larva cell. Future studies should investigate the timing of palpi movements in relation to adult–larva contacts and food delivery, possibly through the use of artificial transparent nests (Turillazzi, 1980).

An alternative hypothesis about the function of AbW is related to its possible long-term effect on the behavior and physiology of larvae. Even if the physiological mechanisms that bias individual development into a worker or reproductive female phenotype are not completely solved, it is supposed that, in *Polistes*, caste determination mainly occurs in the pre-imaginal phase and it is likely related to the nutritional status of larvae (i.e. well-fed female larvae develop into reproductives) (O'Donnell, 1998; Hunt and Amdam, 2005; Hunt et al., 2007). However, according to Brillet et al. (1999) and Jeanne (2009), the substrate-borne vibrations produced by the oscillatory movements in *Polistes* wasps could be seen as signals that advertise the presence of a dominant and breeding individual on the nest, to both maintain hierarchy on the nest and prepare the larvae for their future status as workers. In this way, vibrations could trigger physiological events in larvae driving caste development toward a worker phenotype, as has been demonstrated in *P. fuscatus* with antennal drumming (Suryanarayanan et al., 2011b; Jandt et al., 2017). We did not test long-term effects, but this hypothesis might explain why AbW is more common in dominant than in subordinate individuals and why it is more frequent in the early than in the late phase of the colony cycle (Savoyard et al., 1998; Brillet et al., 1999; Jeanne, 2009).

Overall, our results (i) provide the first direct evidence that the substrate-borne vibrations produced on the nest paper by AbW are perceived by larvae and elicit in them a behavioral response, thus strongly supporting AbW as a signal with a role in adult–larval communication, which is largely corroborated by correlative, but so far no direct, evidence (Brillet et al., 1999; Brennan, 2007; Mignini and Lorenzi, 2015); and (ii) do not support the salivary regulation hypothesis, i.e. no support has been found for an influence of AbW-produced vibrations on the amount of saliva released by larvae, either as an increase or a decrease of it, as previous authors have suggested (Brennan, 2007).

Future studies should use playback to assess any long-term effect of AbW on larval phenotype (behavior, physiology, gene expression) and to evaluate the possible interaction between vibrations and other communication channels (i.e. chemical), specifically testing the possible modulation effect of vibrational signals. Moreover, future studies should assess the possible interplay between substrate-borne vibrations and the physical presence of adult foundresses on the nest. Our observations contribute to the growing body of evidence (Hunt and Richard, 2013; Cervo et al., 2015) that communication channels others than chemicals have an important role in regulating insect social life.

Acknowledgements

We are extremely grateful to Beatrice Boschi and Mara Gessini for their help in data collection, and we thank CBC Europe, Milan, Italy, for their help in developing vibrational equipment. We warmly thank Maria Cristina Lorenzi, Seirian Sumner, Michael Bentley, Adam Devenish and Benjamin Aaron Taylor for their valuable comments on an earlier draft of the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.C., R.C.; Methodology: I.P., A.C., R.N., V.M., R.C.; Software: R.N., V.M.; Validation: R.N., V.M.; Formal analysis: A.C.; Investigation: I.P., A.C.; Resources: V.M., R.C.; Data curation: A.C.; Writing - original draft: A.C.; Writing - review & editing: I.P., A.C., R.N., V.M., R.C.; Visualization: I.P., A.C.; Supervision: R.C.; Project administration: R.C.; Funding acquisition: R.C.

Funding

This research was supported by funds from the University of Florence (to R.C.) and by the European Commission's Horizon 2020 research and innovation programme, under Marie Skłodowska-Curie grant 706208-SocParPhenoEvol to A.C.

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.186247.supplemental

Reference

Barenholz-Paniry, V. and Ishay, J. S. (1988). Synchronous sounds produced by *Vespa orientalis* larvae. *J. Acoust. Soc. Am.* **84**, 841-846.

Barenholz-Paniry, V., Ishay, J. S. and Grossman, Z. (1988). Rhythmic signalling and entrainment in *Vespa orientalis* larve: characterization of the underlying interactions. *Bull. Math. Biol.* **50**, 661-679.

Beani, L. (1996). Lek-like courtship in paper-wasps: a prolonged, delicate, and troublesome affair. In *Natural History and Evolution of Paper-Wasps* (ed. S. Turillazzi and M. J. West-Eberhard), pp. 113-125. Oxford, UK: Oxford University Press.

Brennan, B. J. (2007). Abdominal wagging in the social paper wasp *Polistes dominulus*: behavior and substrate vibrations. *Ethology* **113**, 692-702.

Brillet, C., Tian-Chansky, S. S. and Conte, Y. L. (1999). Abdominal waggings and variation of their rate of occurrence in the social wasp, *Polistes dominulus* Christ. I. Quantitative analysis. *J. Insect Behav.* **12**, 665-686.

Bulmer, M. G. (1983). The significance of protandry in social Hymenoptera. *Am. Nat.* **121**, 540-551.

Burton, P., Gurrin, L. and Sly, P. (1998). Extending the simple linear regression model to account for correlated responses: an introduction to generalized estimating equations and multi-level mixed modelling. *Stat. Med.* **17**, 1261-1291.

Cappa, F., Bruschini, C., Cervo, R., Turillazzi, S. and Beani, L. (2013). Males do not like the working class: male sexual preference and recognition of functional castes in a primitively eusocial wasp. *Anim. Behav.* **86**, 801-810.

Casacci, L. P., Thomas, J. A., Sala, M., Treanor, D., Bonelli, S., Balletto, E. and Schönrogge, K. (2013). Ant pupae employ acoustics to communicate social status in their colony's hierarchy. *Curr. Biol.* **23**, 323-327.

Cassill, D. L. and Tschinkel, W. R. (1995). Allocation of liquid food to larvae via trophallaxis in colonies of the fire ant, *Solenopsis invicta*. *Anim. Behav.* **50**, 801-813.

Cervo, R., Cini, A. and Turillazzi, S. (2015). Visual recognition in social wasps. In *Social Recognition in Invertebrates* (ed. L. Aquiloni and E. Tricarico), pp. 125-145. Cham, ZH: Springer International Publishing.

Cocroft, R. B. and Rodríguez, R. L. (2005). The behavioral ecology of insect vibrational communication. *Bioscience* **55**, 323-334.

Cocroft, R. B., Gogala, M., Hill, P. S. and Wessel, A. (2014). *Studying Vibrational Communication*. Berlin: Springer.

Cohen, J. (1969). *Statistical Power Analysis for the Behavioural Sicences*. New York, NY: Academic press.

Creemers, B., Billen, J. and Gobin, B. (2003). Larval begging behaviour in the ant *Myrmica rubra*. *Ethol. Ecol. Evol.* **15**, 261-272.

Cummings, D. L. D., Gamboa, G. J. and Harding, B. J. (1999). Lateral vibrations by social wasps signal larvae to withhold salivary secretions (*Polistes fuscatus*, Hymenoptera: Vespidae). *J. Insect Behav.* **12**, 465-473.

Dapporto, L. and Palagi, E. (2006). Wasps in the shadow: looking at the pre-hibernating clusters of *Polistes dominulus*. *Ann. Zool. Fennici* **43**, 583-594.

De Groot, M., Čokl, A. and Virant-Doberlet, M. (2011). Search behaviour of two hemipteran species using vibrational communication. *Centr. Europ. J. Biol.* **6**, 756.

Evans, T. A., Inta, R., Lai, J. C. S. and Lenz, M. (2007). Foraging vibration signals attract foragers and identify food size in the drywood termite, *Cryptotermes secundus*. *Insect. Soc.* **54**, 374-382.

Friard, O. and Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* **7**, 1325-1330.

Gamboa, G. J. and Dew, H. E. (1981). Intracolonial communication by body oscillations in the paper wasp, *Polistes metricus*. *Insect. Soc.* **28**, 13-26.

Gamboa, G. J., Wacker, T. L., Scope, J. A., Cornell, T. J. and Shellman-Reeve, J. (1990). The mechanism of queen regulation of foraging by workers in paper wasps (*Polistes fuscatus*, Hymenoptera: Vespidae). *Ethology* **85**, 335-343.

Hager, F. A. and Kirchner, W. H. (2014). Directional vibration sensing in the termite *Macrotermes natalensis*. *J. Exp. Biol.* **217**, 2526-2530.

Harding, B. J. and Gamboa, G. J. (1998). Sequential relationship of body oscillations in the paper wasp, *Polistes fuscatus* (Hymenoptera: Vespidae). *Great Lakes Entomol.* 31, no. 3, article 7. https://scholar.valpo.edu/tgle/vol31/iss3/7

Heimken, C., Aumeier, P. and Kirchner, W. H. (2009). Mechanisms of food provisioning of honeybee larvae by worker bees. *J. Exp. Biol.* **212**, 1032-1035.

Hill, P. S. M. (2008). *Vibrational Communication in Animals*. Cambridge, MA: Harvard University Press.

Hölldobler, B. (1999). Multimodal signals in ant communication. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* **184**, 129-141.

Hölldobler, B., Braun, U., Gronenberg, W., Kirchner, W. H. and Peeters, C. (1994). Trail communication in the ant *Megaponera foetens* (Fabr.) (Formicidae, Ponerinae). *J. Insect Physiol.* **40**, 585-593.

Huang, Z.-Y. and Otis, G. W. (1991). Inspection and feeding of larvae by worker honey bees (Hymenoptera: Apidae): effect of starvation and food quantity. *J. Insect Behav.* **4**, 305-317.

Hubbard, A. E., Ahern, J., Fleischer, N. L., Van der Laan, M., Lippman, S. A., Jewell, N., Bruckner, T. and Satariano, W. A. (2010). To GEE or not to GEE: comparing population average and mixed models for estimating the associations between neighborhood risk factors and health. *Epidemiology* **21**, 467-474.

Hunt, J. H. (1988). Lobe erection behavior and its possible social role in larvae of *Mischocyttarus* paper wasps. *J. Insect Behav.* **1**, 379-386.

Hunt, J. H. (1991). Nourishment and the evolution of the social Vespidae. In *The Social Biology of Wasps* (ed. K. G. Ross and R. W. Matthews), pp. 426-450. Ithaca, NY: Cornell University Press.

Hunt, J. H. (2007). *The Evolution of Social Wasps*. Oxford University Press.

Hunt, J. H. and Amdam, G. V. (2005). Bivoltinism as an antecedent to eusociality in the paper wasp genus *Polistes*. *Science* **308**, 264-267.

Hunt, J. H. and Richard, F.-J. (2013). Intracolony vibroacoustic communication in social insects. *Insect. Soc.* **60**, 403-417.

Hunt, J. H., Baker, I. and Baker, H. G. (1982). Similarity of amino acids in nectar and larval saliva: the nutritional basis for trophallaxis in social wasps. *Evolution* **36**, 1318-1322.

Hunt, J. H., Kensinger, B. J., Kossuth, J. A., Henshaw, M. T., Norberg, K., Wolschin, F. and Amdam, G. V. (2007). A diapause pathway underlies the gyne phenotype in *Polistes* wasps, revealing an evolutionary route to caste-containing insect societies. *Proc. Natl. Ac. Sci. USA* **104**, 14020-14025.

Ishay, J. and Brown, M. B. (1975). Patterns in the sounds produced by *Paravespula germanica* wasps. *J. Acoust. Soc. Am.* **57**, 1521-1525.

Ishay, J. and Ikan, R. (1968). Food exchange between adults and larvae in *Vespa orientalis* F. *Anim. Behav.* **16**, 298-303.

Ishay, J. and Landau, E. M. (1972). *Vespa* larvae send out rhythmic hunger signals. *Nature* **237**, 286-287.

Ishay, J., Motro, A., Gitter, S. and Brown, M. B. (1974). Rhythms in acoustical communication by the oriental hornet, *Vespa orientalis*. *Anim. Behav.* **22**, 741-744.

Ishikawa, Y., Yamada, Y. Y., Matsuura, M., Tsukada, M. and Tsuchida, K. (2011). *Polistes japonicus* (Hymenoptera, Vespidae) queens monopolize ovipositing but are not the most active aggressor in dominant-subordinate interactions. *Insect. Soc.* **58**, 519.

Kaptein, N., Billen, J. and Gobin, B. (2005). Larval begging for food enhances reproductive options in the ponerine ant *Gnamptogenys striatula*. *Anim. Behav.* **69**, 293-299.

Kawatsu, K. (2013). Effect of nutritional condition on larval food requisition behavior in a subterranean termite *Reticulitermes speratus* (Isoptera: Rhinotermitidae). *J. Ethol.* **31**, 17-22.

Kirchner, W. H. (1993). Vibrational signals in the tremble dance of the honeybee, *Apis mellifera*. *Behav. Ecol. Sociobiol.* **33**, 169-172.

Kirchner, W. H., Broecker, I. and Tautz, J. (1994). Vibrational alarm communication in the damp-wood termite *Zootermopsis nevadensis*. *Physiol. Entomol.* **19**, 187-190.

Jandt, J. M., Suryanarayanan, S., Hermanson, J. C., Jeanne, R. L. and Toth, A. L. (2017). Maternal and nourishment factors interact to influence offspring developmental trajectories in social wasps. *Proc. Royal Soc. A* **284**, 20170651.

Jeanne, R. L. (2009). Vibrational signals in social wasps: a role in caste determination. In *Organization of Insect Societies: from Genome to Sociocomplexity* (ed. J. Gadau and J. Fewell), pp. 241-263. Cambridge, MA: Harvard University Press.

Leadbeater, E., Carruthers, J. M., Green, J. P., Rosser, N. S. and Field, J. (2011). Nest inheritance is the missing source of direct fitness in a primitively eusocial insect. *Science* **333**, 874-876.

Lewis, L. A. and Schneider, S. S. (2000). The modulation of worker behavior by the vibration signal during house hunting in swarms of the honeybee, *Apis mellifera*. *Behav. Ecol. Sociobiol.* **48**, 154-164.

Mazzoni, V., Polajnar, J. and Virant-Doberlet, M. (2015). Secondary spectral components of substrate-borne vibrational signals affect male preference. *Behav. Processes* **115**, 53-60.

McGregor, P. K. (1992). *Playback and Studies of Animal Communication*. Springer Science and Business Media.

Mignini, M. and Lorenzi, M. C. (2015). Vibratory signals predict rank and offspring caste ratio in a social insect. *Behav. Ecol. Sociobiol.* **69**, 1739-1748.

Nascimento, F. S., Hrncir, M., Tolfiski, A. and Zucchi, R. (2005). Scraping sounds produced by a social wasp (Asteloeca ujhelyii, Hymenoptera: Vespidae). *Ethology* **111**, 1116-1125.

O'Donnell, S. (1998). Reproductive caste determination in eusocial wasps (Hymenoptera: Vespidae). *Annu. Rev. Entomol.* **43**, 323-346.

Overall, J. E. and Tonidandel, S. (2004). Robustness of generalized estimating equation (GEE) tests of significance against misspecification of the error structure model. *Biom. J.* **46**, 203-213.

Pan, W. (2001). Akaike's information criterion in generalized estimating equations. *Biometrics* **57**, 120-125.

Pereboom, J. J. M., Velthuis, H. H. W. and Duchateau, M. J. (2003). The organisation of larval feeding in bumblebees (Hymenoptera, Apidae) and its significance to caste differentiation. *Insect. Soc.* **50**, 127-133.

Polajnar, J., Eriksson, A., Stacconi, M. V. R., Lucchi, A., Anfora, G., Virant-Doberlet, M. and Mazzoni, V. (2014). The process of pair formation mediated by substrate-borne vibrations in a small insect. *Behav. Processes* **107**, 68-78.

Pratte, M. and Jeanne, R. L. (1984). Antennal drumming behavior in *Polistes* wasps (Hymenoptera: Vespidae). *Ethology* **66**, 177-188.

Queller, D. C., Zacchi, F., Cervo, R., Turillazzi, S., Henshaw, M. T., Santorelli, L. A. and Strassmann, J. E. (2000). Unrelated helpers in a social insect. *Nature* **405**, 784-787.
Reeve, H. K. (1991). *Polistes*. In *The Social Biology of Wasps* (ed. K. G. Ross and R. H. Matthews), pp. 99-148. Ithaca, NY: Cornell University Press.
Röhrig, A., Kirchner, W. H. and Leuthold, R. H. (1999). Vibrational alarm communication in the African fungus-growing termite genus Macrotermes (Isoptera, Termitidae). *Insect. Soc.* **46**, 71-77.
Savoyard, J. L., Gamboa, G. J., Cummings, D. L. D. and Foster, R. L. (1998). The communicative meaning of body oscillations in the social wasp, *Polistes fuscatus* (Hymenoptera, Vespidae). *Insect. Soc.* **45**, 215-230.
SPSS, I. (2011). *IBM SPSS statistics for Windows, version 20.0*. New York: IBM Corp.
Suryanarayanan, S. and Jeanne, R. L. (2008). Antennal drumming, trophallaxis, and colony development in the social wasp *Polistes fuscatus* (Hymenoptera: Vespidae). *Ethology* **114**, 1201-1209.
Suryanarayanan, S., Hantschel, A. E., Torres, C. G. and Jeanne, R. L. (2011a). Changes in the temporal pattern of antennal drumming behavior across the *Polistes fuscatus* colony cycle (Hymenoptera, Vespidae). *Insect. Soc.* **58**, 97-106.
Suryanarayanan, S., Hermanson, J. C. and Jeanne, R. L. (2011b). A mechanical signal biases caste development in a social wasp. *Curr. Biol.* **21**, 231-235.
Turillazzi, S. (1980). Use of artificial nests for rearing and studying *Polistes* wasps. *Psyche* **87**, 131-140.
Turillazzi, S., Perito, B., Pazzagli, L., Pantera, B., Gorfer, S. and Tancredi, M. (2004). Antibacterial activity of larval saliva of the European paper wasp *Polistes dominulus* (Hymenoptera, Vespidae). *Insect. Soc.* **51**, 339-341.
Vander Meer, R. K. and Alonso, L. E. (1998). Pheromone directed behavior in ants. In *Pheromone Communication in Social Insects* (ed. R. K. Vander Meer, M. Breed, M. Winston and K. E. Espelie), pp. 159-192, Oxford, UK: Westview Press.
Vander Meer, R. K., Breed, M. D., Espelie, K. E. and Winston, M. L. (1998). *Pheromone Communication in Social Insects. Ants, Wasps, Bees and Termites*. Boulder, CO: Westview Press.
Visscher, P. K., Shepardson, J., McCart, L. and Camazine, S. (1999). Vibration signal modulates the behavior of house-hunting honey bees (*Apis mellifera*). *Ethology* **105**, 759-769.
West-Eberhard, M. J. (1969). The social biology of polistine wasps. *Misc. Publ. Mus. Zool. Univ. Mich.* **140**, 1-101.
West-Eberhard, M. J. (1986). Dominance relations in *Polistes canadensis* (L.), a tropical social wasp. *Monit. Zool. Ital.* **20**, 263-281.

Supplementary materials

Materials and methods

Feature	Colony phase	Control	White Noise	Abdominal Wagging	Kruskal Wallis test	sig.	N
Number of Cells	**Pre-emergence**	46.36±13.76	39.00±17.52	41.73±15.92	X^2=2.600	p=0.272	11 vs 11 vs 11
	Post-emergence	81.56±56.01	74.22±36.34	76.89±55.30	X^2=0.292	p=0.864	9 vs 9 vs 9
Number of Pupae	**Pre-emergence**	5.00±5.85	3.18±5.06	3.55±3.61	X^2=1.590	p=0.451	11 vs 11 vs 11
	Post-emergence	17.56±25.96	10.44±11.60	17.55±18.88	X^2=0.585	p=0.746	9 vs 9 vs 9
Number of Larvae	**Pre-emergence**	16.27±8.27	16.36±8.23	18.55±8.81	X^2=0.502	p=0.788	11 vs 11 vs 11
	Post-emergence	23.11±9.74	18.22±5.54	23.11±7.46	X^2=2.836	p=0.805	9 vs 9 vs 9
Number of females	**Pre-emergence**	2.73±1.55	1.82±0.87	2.18±1.32	X^2=2.213	p=0.331	11 vs 11 vs 11
	Post-emergence	14.67±10.82	10.44±4.88	10.00±5.39	X^2=0.434	p=0.805	9 vs 9 vs 9

Table S1 Comparison of demographic features of colonies allocated to the three treatments. Colonies allocated to the different experimental groups did not differ for any demographic parameter considered.

	AbW performed by foundresses			**AbW track**		
	Mean ± SD	**minimum value**	**Maximum value**	**Mean ± SD**	**minimum value**	**Maximum value**
Duration of event (seconds)	0.61 ± 0.41	0.04	2.61	0.91 ± 0.05	0.47	1.10
Dominant frequency (Hz)	48.10 ± 26.59	32	128	69.82 ± 27.29	64	192
Number of pulses	7.27 ± 4.26	2	31	11.79 ± 0.53	11	13
Duration of pulses (seconds)	0.03 ± 0.01	0.004	0.064	0.03 ± 0.01	0.015	0.071

Table S2 Temporal and spectral parameters of AbW performed by foundresses, as recorded in a preliminary experiment on 7 colonies, and features of AbW playback track as re-recorded on the nests on which it was played back.

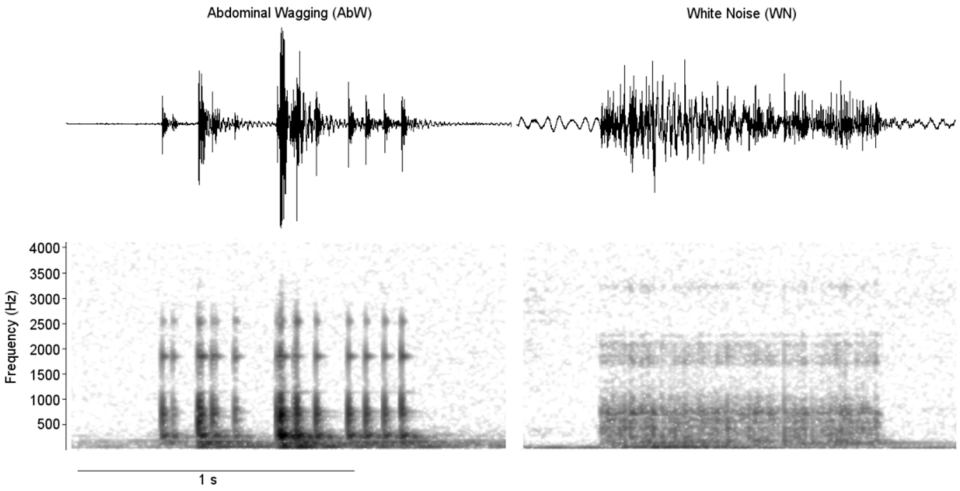

Figure S1. Oscillogram (above) and spectrogram (below) of the two playback tracks of AbW (left) and WN (right).

Experimental apparatus

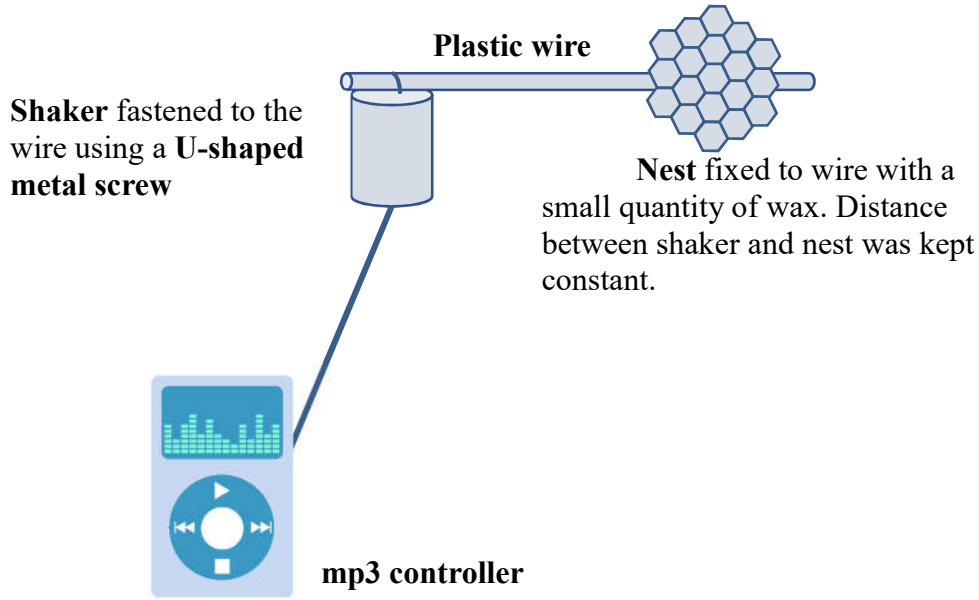

Figure S2 Experimental apparatus used to play back vibration tracks on the nest.

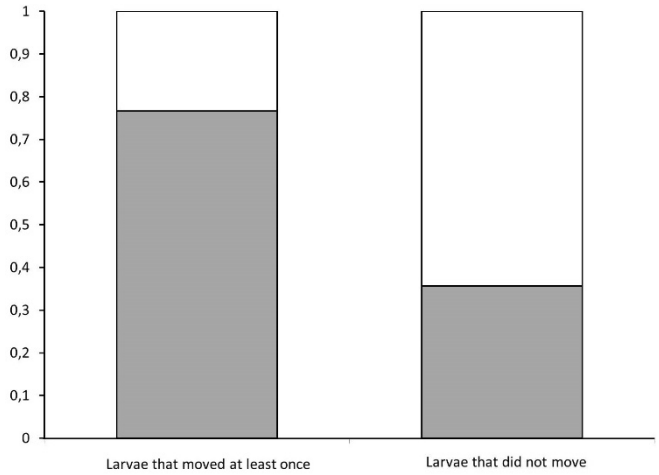

Figure S3. Larvae that performed at least one body movement were more likely to receive at least one food delivery than larvae that did not (Chi-square corrected for continuity=5.22, df=1, p=0.0223). The histogram reports that percentage of larvae that receive a food visit (grey) in the two groups. Data from an additional experiment, 94 larvae from 13 colonies were observed during the feeding context for 15 minutes each.

Behavioural response experiment					
Season	**Larval size**	**Treatment**			**Total**
		C	**WN**	**AbW**	
Pre-emergence	Medium	18	13	23	54
	Large	13	22	14	49
	Total	*31*	*35*	*37*	***103***
Post-emergence	Medium	11	7	6	24
	Large	25	24	27	76
	Total	*36*	*31*	*33*	***100***
Total	Medium	*29*	*20*	*29*	*78*
	Large	38	46	41	125
	Total	***67***	***66***	***70***	***203***
Saliva release experiment					
Season	**Larval size**	**Treatment**			**Total**
		C	**WN**	**AbW**	
Pre-emergence	Medium	27	17	16	*60*
	Large	18	21	24	*73*
	Total	*45*	*38*	*40*	***123***

Post-emergence	Medium	10	18	12	*40*
	Large	43	35	41	*119*
	Total	*53*	*53*	*53*	***159***
Total	Medium	37	35	28	*100*
	Large	61	56	65	*182*
	Total	***98***	***91***	***93***	***282***

Table S3. Sample sizes of larvae of each category tested in the behavioural response and saliva release experiments.

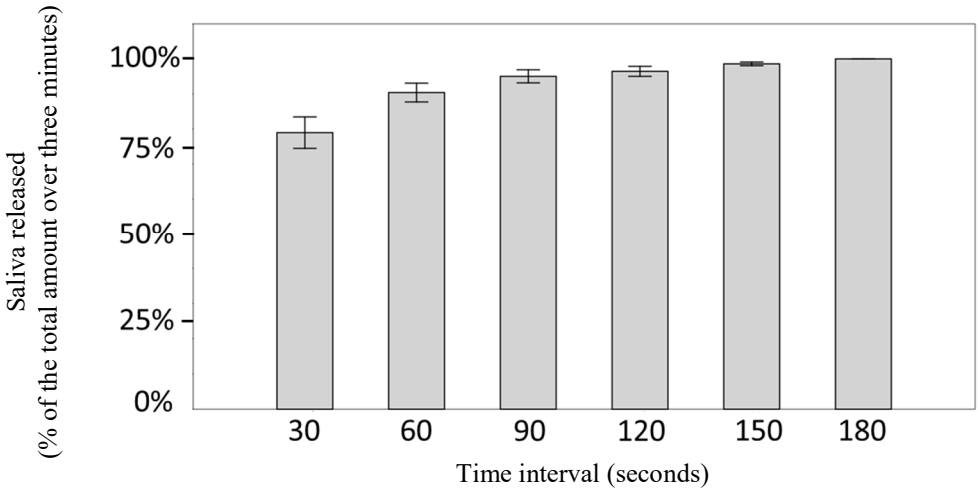

Figure S4 Percentage of saliva (mean ± standard error) released in 30, 60, 90, 120, 150 and 180 seconds without vibrational stimulation.

Results

body movements			
Source	**Type III Wald Chi-Square**	**df**	**p-value**
(Intercept)	0.367	1	0.545
Treatment	**44.409**	**2**	**<0.001**
Colony phase	**4.755**	**1**	**0.029**
Larval size	**4.274**	**1**	**0.039**
Colony phase x Treatment	3.712	2	0.156
Treatment x Larval size	1.232	2	0.54
Colony phase x Larval size	0.026	1	0.873
palpi movements			
Source	**Type III Wald Chi-Square**	**df**	**p-value**
(Intercept)	**68.368**	**1**	**<0.001**
Treatment	0.068	2	0.967
Colony phase	1.432	1	0.231
Larval size	3.540	1	0.060
Colony phase x Treatment	0.530	2	0.767
Treatment x Larval size	0.932	2	0.627
Colony phase x Larval size	0.067	1	0.796

Table S4 Influence of treatment, colony phase and larval size on binary logistic distribution of body movements (above) and palpi movements (below). Results from Generalized Estimating Equation. Significant explanatory variables in bold.

Source	**Type III Wald Chi-Square**	**df**	**p-value**
(Intercept)	**610.074**	**1.000**	**<0.001**
Treatment	0.250	2.000	0.883
Colony phase	**17.180**	**1.000**	**<0.001**
Larval size	**11.251**	**1.000**	**0.001**
Treatment x Colony phase	0.472	2.000	0.790
Colony phase x Larval size	2.715	1.000	0.099
Treatment x Larval size	2.887	2.000	0.236

Table S5 Influence of treatment, colony phase and larval size on amount of larval saliva release in 60 seconds. Results from Generalized estimating equation. Significant explanatory variables in bold.

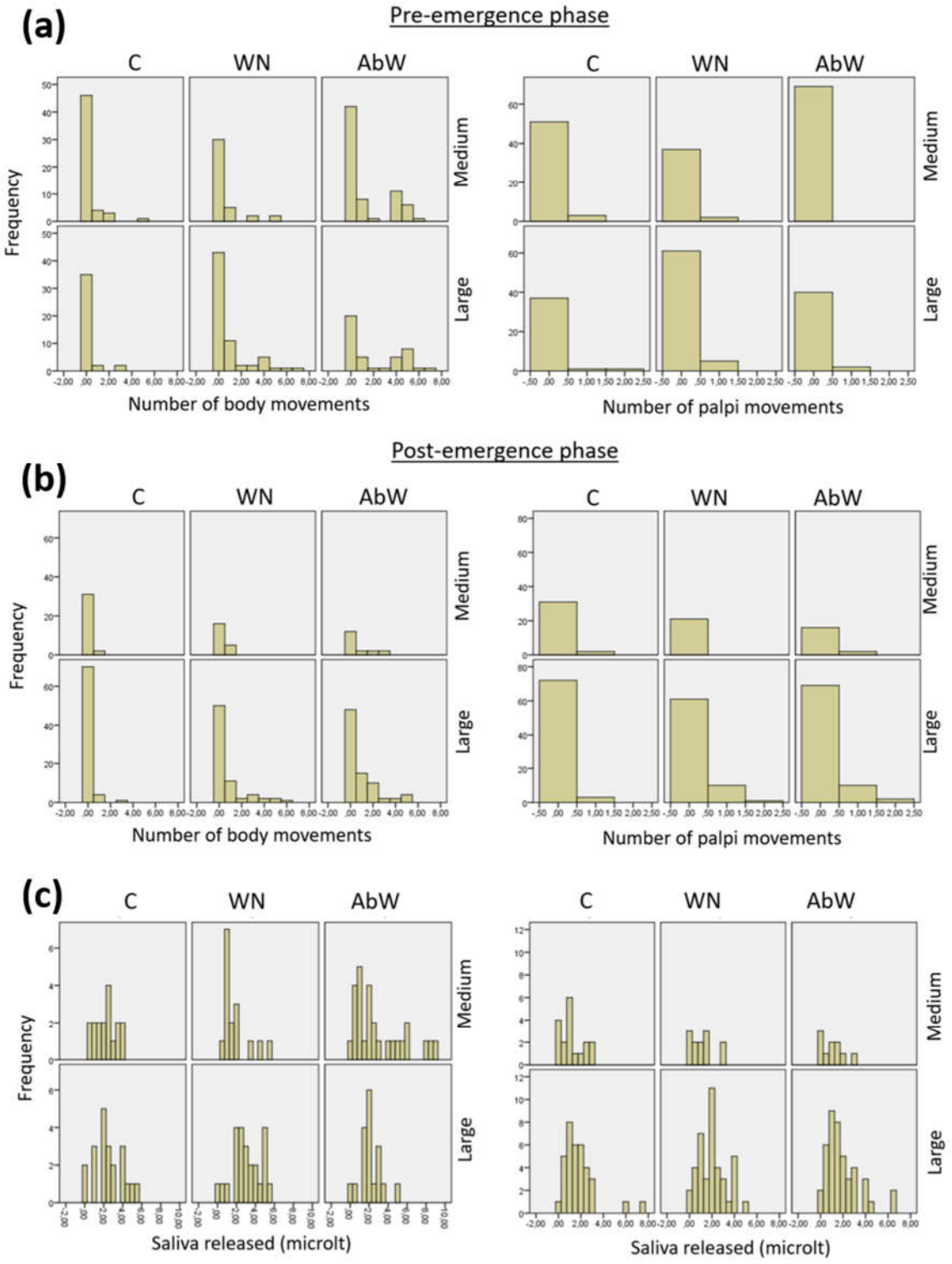

Fig. S5. Frequency histogram of the values assumed by (a) the number of body and palpi movements according to treatment and larval size, for the pre-emergence phase, (b) the number of body and palpi movements according to treatment and larval size, for the post-emergence phase and (c) by the amount of saliva released (microlt) according to treatment and larval size, for the pre-emergence period, left, and for the post-emergence period, right.

Audio 1. Audio track of Abdominal wagging used for playback experiments

Click here to Download Audio 1

Audio 2 Audio track of White noise used for playback experiments

Click here to Download Audio 2

Movie 1. An example of the behavioural responses recorded in larvae

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