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COORDINATORE Prof. Alberto Ugolini

Beyond chemicals: neglected communication
modalities in paper-wasp societies

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Dottorando

Dott. Pepiciello Irene

Tutore

Prof. Cervo Rita

Coordinatore

Prof. Ugolini Alberto

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1. Abstract

Social life requires a complex and efficient communication system. Traditionally, chemical communication is considered the most important sensory channel in insects societies. However, in the last decades, visual and substrate-borne vibrational communication have inspired a growing interest. In order to understand if these understudied sensory channels could be used in fundamental aspects of social life, such as nestmate recognition, dominance hierarchies and adult-brood interactions, I used the primitively eusocial paper wasp *Polistes dominula*, whose features of nest structure and the body coloration pattern are suitable to vibrational and visual communication. The main results of this work are: *i)* *P. dominula* wasps can use both visual and chemical signals in nestmate recognition according to the colony social environment; *ii)* substrate-borne vibrations produced by adult females evoke a measurable behavioral response in larvae, which thus provides evidence that vibrations can play a role as signal; *iii)* the intensity of vibrations produced by different females in associative foundations is related to individual position in the dominance hierarchy position and, from the physiological point of view, to ovary development, suggesting that such feature could vehicle individual information; *iv)* finally, the study of vibrations produced by *Polistes sulcifer*, the obligate social parasite of *P. dominula*, suggests that the parasite could exploit the vibrational host communication system to get integrated into the host colony.

Overall, the results of this work show that communication modalities other than the chemical one can play a crucial role in regulating insect social life.

2. Introduction

Insects social life is characterized by complex social interactions that require a sophisticated and efficient communication system that allows, among other things, to recognize fertility status, caste membership, nestmate from non-nestmate and gender (Wilson 1971, van Zweden and d’Ettorre 2010, Cervo et al. 2015).

Commonly, in dark and crowded colonies of social insects, chemical communication plays a principal and pivotal role (Wilson 1971, Howard & Blomquist 2005, Richard & Hunt 2013).

However, social communication can occur via different sensory channels that can combine with each other in a complex communication system (Hölldobler 1999, Hunt & Richard 2013, Cervo et al. 2015). Indeed, visual and vibrational signals also can provide information quickly and in some situations, as in agonistic ability signaling, nest-mate recognition or alarm signaling, these sensory channels can be used by social insects in addition or replacing chemical communication (Hölldobler 1999, Hunt & Richard 2013, Baracchi et al. 2015, Cervo et al. 2015). A good model to study multimodal communication is represented by primitively eusocial wasps of *Polistes* genus. Indeed, the social life of these wasps occurs in small uncovered nests where sunlight allows inhabitants to use visual signals (Cervo et al. 2015); moreover, these combs are built with paper material that permits the transmission of substrate-borne vibrations through the colony structure (Brennan 2007) by adding this potential mechanical communication mode to chemical and visual ones. So, in this thesis I have investigated if and how the two above-mentioned neglected communication channels can play a role in regulating social life in *Polistes dominula*, a model species for studies on social insects chemical communication.

It is well known that chemical compounds are involved in many aspects of *Polistes* life: from alarm communication with highly volatile compounds able to evoke an aggressive response in nestmates against a potential threat to the colony (Bruschini et al. 2006), to communication about fertility and social status with non-volatile compounds that cover wasp bodies (i.e. epicuticular mixture of hydrocarbons) (Sledge et al. 2001a, Dapporto et al. 2005, Dapporto et al. 2007). Moreover, in *P. dominula*, as in many other social insects, the same blend of cuticular hydrocarbons (mainly saturated and unsaturated hydrocarbons with a primary and main function to reduce both internal water loss and parasite and pathogens entrance, Blomquist & Dillwith 1985) are involved in nestmate recognition (Lorenzi et al. 1996, Howard & Blomquist 2005, Dani 2006, Bruschini et al. 2010, van Zweden & d’Ettorre 2010), a crucial aspect of social life that allows to maintain the colony integrity and to defend colonial resources that could be exploited by non-nestmate conspecifics or heterospecific individuals (d’Ettorre and Lenoir 2010). Such complex blend provides a chemical signature shared by members of the same colony but that differs among

individuals belonging to different colonies (see Lorenzi et al. 1996, Dani 2006, Bruschini et al. 2010). This colony chemical signature is learned by each wasp from its own colony immediately after the emergence (Gamboa et al. 1986, Gamboa 1996, 2004). Once formed, this reference template, is used by wasps to compare the chemical signature of each individual they meet throughout their life. This comparison allows each wasp to discriminate nestmate (when template matches with chemical phenotype) from non-nestmate individuals (when template and chemical phenotype do not match) (Gamboa 2004, Lorenzi et al. 1996, Dani 2006, Signorotti et al. 2015).

In addition to this massive use of chemicals in *Polistes* life and in social recognition, in the last 15 years, an increasing number of studies has highlighted the presence of visual communication in this genus (reviewed by Cervo et al. 2015). In *P. fuscatus*, for instance, facial and abdominal markings are used for individual nestmate recognition (Tibbetts 2002). Furthermore, in North American population of *P. dominula*, in which the shape and the number of black spots on clypeus are related with rank position and individual size, such color facial patterns of clypeus can be used as quality signal (Tibbetts & Dale 2004). Furthermore, the variability in coloration pattern of clypeus in this species (Tibbetts and Dale 2004, Cervo et al. 2008, Tibbetts 2006, Tibbetts et al. 2011, Green and Field 2011, Green et al. 2013, Zanette and Field 2009) and the importance of visual cues in this genus (reviewed by Cervo et al. 2015) suggest that visual cues could play a role in *P. dominula* nestmate recognition in additions to chemical ones provided by cuticular hydrocarbons. The drastically change in inhabitants number during colony development suggests a plastic use of communication channels. In the pre-emergence phase, when only few individuals are present on the nest (co-foundresses that associated together in spring to start a new colony) and repeated encounters between a small number of colony members occur, allowing to learn their visual pattern, recognition mechanism could be based mainly on visual cues. Moreover, visual cues might be advantageous over chemicals because the first ones, by not requiring a contact between individuals, allow a faster processing and a faster response (Baracchi et al. 2015, Cervo et al. 2015).

Then, with the growth of the colony, when many workers are emerged and the increase in colony members number reduces the reliability of visual cues, we can expect that cuticular hydrocarbons assume a primary role in nestmate recognition as described and stressed in a plenty of previous studies (see Gamboa 1996, 2004, Dani et al. 2001, Dani 2006, Bruschini et al. 2010).

In this framework, by separating experimentally visual and chemical cues of nestmates and non-nestmate individuals and presenting them alone and in coherent or in mismatch combination to resident females of *P. dominula* I investigated if chemical and visual cues assume a different relative importance according to the colony social environment (i.e. pre-emergence and post-

emergence colony phase) by highlighting a plasticity in the use of different communication channels (Chapter 3).

In *Polistes* pre-emergence phase, when one or few adult females live in “low-noise” colony, another communication channel can be used: substrate-borne vibrations (Brennan 2007, Jeanne 2009). Despite that since the beginning of twentieth century it has been speculated an involvement of the substrate-borne vibrations in animal communication (Cocroft et al. 2014), only in the last years new technologies and renewed interest in sensory channels alternative to the chemical one have highlighted that the vibrational signals are widespread in social insects (Hunt & Richard 2013). Vibrational communication has been reported in all groups of social insects (bees, wasps, ants and termites) (reviewed in Hunt & Richard 2013), however, it has been pointed out how wasps of the genus *Polistes* largely use vibrations for communication (Jeanne 2009). Thus, also the substrate-borne vibrational communication is attracting a new and growing interest in the study of social communication in this genus where some behaviors performed by females are able to generate vibrations that are transmit along comb structure (Pratte & Jeanne 1984, West-Eberhard 1986, Gamboa & Dew 1981, Savoyard et al. 1998, Brillet et al. 1999, Brennan 2007, Suryanarayanan et al. 2011, Mignini & Lorenzi 2015). These behaviors are performed during both adult-larvae and aggressive adult-adult interactions. Adult-larvae interactions are represented by nest inspection, with or without food distribution and are performed by adult females (West-Eberhard 1969, 1986, Gamboa & Dew 1981, Savoyard et al. 1998, Brillet et al. 1999).

An intriguing hypothesis is that the vibrations produced by the adult females on the nest have effects on both adults and larvae, by playing a crucial role both in social relationships and in caste determination fate of developing larvae (Brillet et al. 1999, Jeanne 2009). According to Jeanne (2009), vibrations and dominance interactions “are part of the same functional continuum as physical attacks by dominant individuals on subordinates” by signaling the presence of a dominant and reproductive female on the nest and by maintaining the hierarchy among foundresses. At the same time, vibrations could affect developing larvae with a long-term effect, by preparing them to their future status of workers (West-Eberhard 1969, Brillet et al. 1999, Jeanne 2009, Suryanarayanan et al 2011, Mignini & Lorenzi 2015, Jandt et al. 2017). In the American species, *P. fuscatus*, it has been found that low frequencies vibrations like those produced by females on the nest, affect larval growing rates and drive larval development toward worker phenotype (Suryanarayanan et al 2011, Jandt et al. 2017). Despite these direct evidences reported in other *Polistes* species suggest that substrate-borne vibrations could play a crucial role also in a communicative system of *P. dominula*, only few correlative evidences regarding their

role have been reported for this species, in which only one behavior able to generate vibrations (called abdominal wagging) has been described and analyzed (Brillet 1999, Brennan 2007).

Another non-mutual exclusive hypothesis has been proposed (*short-term effect hypothesis*): vibrations could represent a signal able to regulate the release larval saliva, which is rich in nutrients. It has been suggested that such vibrations could prepare larvae to receive food by decreasing salivary secretion (Cummings et al. 1999, Brennan 2007, Suryanarayanan & Jeanne 2008, Jeanne 2009) or, alternatively, could represent a request of nutrient saliva to larvae by adults, thereby increasing the amount of saliva released by larvae (Hunt et al. 1982, Hunt 1991, Savoyard et al. 1998). However, until now, no direct evidence has been reported so far about the short-term effect of abdominal wagging.

In this thesis, I investigated the features and the possible communicative role of vibrations produced by adult females in *P. dominula* nest. For this purpose, I used a Laser Doppler vibrometer that, contrary to accelerometer used until now (Brennan 2007), allows us to record vibrations produced on the nest by adult females without altering them. Moreover, thanks to an electro-magnetic shaker I played back abdominal wagging vibrations on the nest in the absence of chemical or visual stimuli (adults on the nest) in order to evaluate the short-term larval response to adults' vibrations.

To shed light on the role of vibrations of dominant individuals in the *Polistes* society I investigated what happens in the well-known parasite-host pair *Polistes sulcifer* – *P. dominula* (Cervo & Dani 1996, Cervo 2006). *P. sulcifer* is an obligate social parasite i.e. a species that has lost the nest-building ability and lacks its own worker force (Cervo 2006). The parasite female, after fighting with *P. dominula* foundresses to invade their colony (before the emergence of workers), usurps the higher dominance rank position in the colonial hierarchy by eliminating or submitting the former dominant queen (Turillazzi et al 1990). Once on the nest, the parasite female behaviorally and chemically (Turillazzi et al 1990, Sledge et al. 2001b, Dapporto et al. 2004) imposes herself as dominant individual by integrating in host hierarchy and by assuming the cuticular profile of dominant host females (Dapporto et al. 2004). Furthermore, parasite needs the host workers to rear her brood, which is represented exclusively by reproductive individuals. Interesting, as soon as the parasite usurps the host nest, she performs a peculiar behavior consisting in drumming the abdomen on the nest and this behavior seems able to produce substrate-borne vibrations (Cervo 1990, 2006). It is possible that the parasites communicate their dominant and central role in the usurped colonies, both to host adults and larvae, also by exploiting the vibrational system of the host. Indeed the parasite could exploit the vibrational communication system to get integrated into the host colony and possibly also drive the host larval

development toward the worker phenotype. In order to disentangle the importance of rank from species effect, I recorded vibrations produced by host females, dominant social parasite females, as well as by host females after usurpation and compare them (Chapter 4).

Since the vibrations recorded on queen-right and usurped *P. dominula* colonies suggest that oscillatory behaviors could represent a way to establish a dominant and central role on host colony, I investigated the features of substrate-borne vibrations as potential signal of dominance and quality in *P. dominula* hierarchy on queen-right colonies. I recorded, analyzed and compared vibrations generated by dominant and subordinate foundresses in associative colonies of *Polistes dominula* and tested the influence of morpho-physiological parameters (i.e. size, ovary development) on temporal and spectral features of vibrational signals (Chapter 5). If vibrations are used as honest signal able to furnish information about individual that perform abdominal wagging, we expected to find a relation between foundresses morphological and/or physiological features and abdominal wagging vibrational parameters.

Even if abdominal wagging seems be used as dominance signal, it not excludes that vibrations could play a role in short-term communication between adult-larvae, as previously hypothesized. So, I evaluated the behavioral response of larvae to artificial vibrational stimuli that simulate the females vibrations. In this way I tested if larval behavior changes immediately after abdominal wagging vibrations, in order to assess whether larvae perceived the vibration stimulus produced by such specific adult behavior in the absence of other stimuli (i.e. visual or chemical ones). Furthermore, I tested if vibrations can regulate larval saliva secretion as expected from the *salivary release modulation hypothesis* (Jeanne 2009), by measuring the amount of saliva released immediately after playback in two different periods of the colony cycle (Chapter 6).

In conclusion, in this thesis I have investigated if, in the model species *P. dominula*, visual and vibration signals can play a role in social life and how they could combine and/or replace chemicals as a growing body of evidence suggested during the last years (Tibbetts & Dale 2004, Brillet et al. 1999, Brennan 2007, Hunt & Richard 2013, Cervo et al. 2015).

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3. Sight in a clique, scent in society: plasticity in the use of nestmate recognition cues along colony development in the social wasp *Polistes dominula*

Alessandro Cini^{1,2§}, Federico Cappa¹, Irene Pepiciello¹, Leonardo Platania¹, Rita Cervo¹

¹Università di Firenze - Dipartimento di Biologia; Via Madonna del Piano 6, 50019 Sesto Fiorentino (FI), Italy

²Centre for Biodiversity and Environment Research, University College London, Gower Street, London WC1E 6BT

[§]Corresponding author: Alessandro Cini, mail: cini.ales@gmail.com

Abstract

Nestmate recognition, i.e. the ability to discriminate nestmates from foreign individuals, is a crucial feature of insect societies and it has been traditionally considered to be predominantly based on chemical cues. Recent empirical evidence however suggests a relevant plasticity in the use of different communication channels according to cues availability and reliability in different contexts. In particular, visual cues have been shown to influence various types of social recognition in several social insects, but their role in nestmate recognition is still under-investigated. We tested the hypothesis of plasticity in the use of visual and chemical recognition cues in the primitively eusocial wasp *Polistes dominula*, in which the availability and reliability of recognition cues varies across the colony cycle. Indeed, before the emergence of workers, *P. dominula* colonies are rather small (one to few individuals), and the variability in the facial pattern might allow resident wasps to use visual cues for nestmate recognition. After workers emergence, the increase in the number of colony members reduces the reliability of visual cues, thus leaving chemical cues as the most reliable nestmate recognition cues. We thus predict a differential use of chemical and visual cues along colony life. We experimentally separated visual and chemical cues of nestmates and non-nestmates and presented them alone or in combination (with coherent or mismatched cues) to resident wasps to test which communication channel was used in the two stages and, in case, how visual and chemical cues interacted. Our results show, for the first time in a social insect, the differential use of visual and chemical cues for nestmate recognition during colony development, which supports the hypothesis of a plastic, reliability-based use of recognition cues in this species according to the different colonial context.

Keywords: cuticular hydrocarbons, multimodal communication, paper wasps, familiar recognition, phenotypic plasticity

Introduction

Social organization relies upon social recognition, which is the ability of individuals to distinguish among the individuals they encounter and to bias their behavior accordingly, i.e. responding with an adaptive behavior towards the appropriate individual (Ward & Webster 2016). Social recognition thus plays a crucial role in regulation of social interactions within animal groups, by shaping parent-offspring interactions, competitive aggression, mate choice and cooperative behaviors (Waldman 1988, Gherardi et al. 2012, Aquiloni & Tricarico 2015). Eusocial insects, such as ants, wasps, termites and bees, live in complex societies that represent pinnacles of social evolution and whose organization relies on sophisticated forms of social recognition, such as the ability to recognize caste membership, dominance and fertility status, nestmate from non-nestmate and gender (Wilson 1971, van Zweden and d’Ettorre 2010, Cervo et al. 2015).

Nestmate recognition (hereafter NMR), i.e. the ability to discriminate nestmates from non-nestmates, is the quintessential form of social recognition that occurs in insect societies (d’Ettorre and Lenoir 2010). Social insect colonies are rich in resources that conspecific and heterospecific individuals may exploit: nests are costly to produce and advantageous in the protection they provide, colonies are full of harmless and meaty brood, and workers efficiently provide alloparental care that might be selfishly exploited. Many species across the whole range of animal kingdom indeed benefit from exploiting social insects colonies at various extents, from predation to social parasitism (Fürst et al. 2011). NMR evolved to allow colony members to recognize and accept each other while strongly repelling potentially dangerous intruders, thus allowing to protect the colony and direct altruistic acts towards related recipients (Hamilton 1987).

NMR occurs through a process of phenotype matching that involves the perception of a label carried by encountered individual and the comparison of this label with an internal reference (template), i.e. a neural representation of the trait stored within the evaluator peripheral and central nervous system (Crozier and Pamilo 1996, Leonhardt et al. 2007, d’Ettorre and Lenoir 2010, Signorotti et al. 2015). The response of the evaluator depends on how well the label matches the template (van Zweden and d’Ettorre 2010), with the aggressive response triggered when the mismatch exceeds a certain threshold (Reeve 1989). Decades of research convincingly demonstrated that colony identity is mainly encoded in a chemical code based on variations in the blend of cuticular hydrocarbons (CHCs) (Howard and Blomquist 2005, Blomquist and Bagnères 2010). Typically, colonies of a given species have a qualitatively similar CHCs profile which differs in the relative amounts of each compound (Lorenzi et al. 1996, Dani 2006, Bruschini et al. 2010, van Zweden & d’Ettorre 2010).

CHC blends have several advantages as NMR cues compared to other potential cues pertaining to different sensory modalities. First, the CHC blend usually entails dozens to hundreds compounds, which vary in their relative abundance across colonies, so that the signal arising from such a complex mixture can be highly informative about colony membership (van Zweden & d’Ettorre 2010, Sturgis & Gordon 2012). Then, CHC blend profile is highly influenced by the environment (e.g. by diet, Liang & Silverman 2000, Buczkowski et al. 2005) and CHC can be exchanged through social contact, which makes the CHC signal highly flexible, thus enabling to keep the colony signature updated in a continuously changing environment (Richard & Hunt 2013).

While it has been repeatedly shown that CHCs are the main cues used in NMR (reviewed in van Zweden & d’Ettorre 2010, Blomquist & Bagnères, 2010) recent experimental evidence revealed that olfaction might be coupled with, or even overcome by, other sensory modalities, such as vision (Cervo et al. 2015). Indeed, in a tropical hover wasp species characterized by small and flexible societies (*Parischnogaster flavolineata*), colony members are able to perform NMR using individual facial patterns in addition to chemical cues, and in case of contrasting information visual cues are preferred over chemical ones (Baracchi et al. 2013, 2015).

The importance of visual cues in social insects recognition remained overlooked for many decades. The last 15 years of researches, especially in paper- and Stenogastrinae wasps, provided strong empirical evidence about the use of visual cues in several forms of social recognition inside and outside social insect colonies, both in the intraspecific and interspecific context (Cervo et al. 2015). Wasps do indeed show remarkable variation in the color patterning of faces and abdomen and the use of visual cues in social recognition has been shown for almost all species (even if few) investigated so far, which suggests that this ability could be widely widespread in social wasps (reviewed in Cervo et al. 2015).

Despite the potentially smaller informative content of visual cues compared to chemical ones (but see Baracchi et al. 2016) and their static nature (individual color patterning remains stable after emergence, while CHCs blend is continuously updated), visual cues might be advantageous over chemical ones to enable NMR as they require less time to be processed and they do not require contact or close distance, thus enabling a faster NMR decision. Indeed, when assessing a potential intruder, colony members are faced with a tradeoff between speed and accuracy of recognition and, depending on the context, speed might be prioritized over accuracy (Chittka et al. 2009; Baracchi et al. 2015). We can thus predict visual cues to be mostly used in species characterized by small colonies, where repeated encounters with a low number of colony members might allow to learn their visual pattern through a familiarization process. Given that

small insect societies are indeed fairly common in many social insect groups, the use of visual cues in NMR potentially involves many species, especially in the primitively eusocial taxa which represent an interesting experimental window on the evolution of sociality (Rehan & Toth 2015).

Our current understanding of the cues underlying NMR in insect societies thus suggest an association between the sensory channel use for NMR and colony size. Chemicals might be preponderant in large societies, such as in honeybees and many ant species, where visual cues could not clearly be reliable, while visual cues might be involved (together or replacing chemicals) in small societies with variable visual cues (such as those of paper-wasps). In some insect species however, such as independent founding wasps, colony size dramatically changes throughout the colonial development, passing from few to hundreds colony members (Reeve 1991). A compelling question, so far unanswered, is thus to what extent a species is able to plastically shift from using cues of one sensory modalities (e.g. visual) to those of another one (e.g. chemical) during the colonial development. In other words, we wonder if the sensory modality/ies used for NMR is hardwired within the species behavioral repertoire or can change according to availability and reliability of cues in different colonial phases. Answering this question has the potential to document an unexpected and yet undocumented level of plasticity in insect communication and to provide an experimental model system for future studies regarding cognitive abilities of social insect mini-brains.

Here we tested the hypothesis of plasticity in the use of visual and chemical recognition cues according to their reliability as NMR cues in the primitively eusocial wasp *Polistes dominula*.

P. dominula is a temperate paper wasp species whose small colony size and phenotypic plasticity made a model organism for social evolution and communication studies (Pardi et al. 1948, Pardi 1996, Jandt et. al 2014, Dani 2006, Cervo et al. 2015) and, thanks to the recent release of its sequenced genome, also for *omic* studies (Standage et al. 2016). *P. dominula* species also represents a good model to test the existence of plasticity in the use of visual and chemical cues according to their availability and reliability, as *i*) both chemical and visual cues are known to be used in several forms of social recognition, and *ii*) availability and reliability of recognition cues varies across the season (Dani 2006, Cervo et al. 2015).

NMR in *P. dominula* is evident, with non-nestmate who highly repelled (Dani et al. 2001) and a clear variation in CHCs signature among different colonies (Bruschini et al. 2011). On the contrary, facial color patterns (which are widely variable in this species and consists in variable yellow clypeus that can have one or more black spots, with variable size and shape, or no black spots at all) are used for several social recognition forms, such as signaling of dominance status and agonistic abilities (e.g. Tibbets & Dale 2004, Tibbets & Lindsay 2008), gender recognition

(Cappa et al. 2016) and possibly species recognition (Cini et al. 2015, Cervo et al. 2015) but they have never been shown to allow NMR (Cervo et al. 2015).

Here, we experimentally separated visual and chemical cues of nestmates and non-nestmates *P. dominula* wasps and presented them alone or in combination (with coherent or mismatched cues) to resident wasps in NMR behavioral trials. We aimed to test which communication channel was used in the two different moments of colonial cycle (at the beginning of colony cycle when colonies are rather small and later, after the emergence of workers, when colony size dramatically increases) and, in case, how visual and chemical cues interacted. Our prediction was that a differential use of chemical and visual cues occurs along colony life, with visual cues used only, if ever, in the pre-emergence period, while chemical used in both periods. Our results show, for the first time in a social insect to our knowledge, a differential use of chemical and visual cues along the colony cycle and provide the first experimental proof that in this species visual cues, in addition to chemical cues, are used to recognize nestmate from non-nestmate.

Materials and Methods

Animal collection and laboratory rearing

For the first experiment (pre-emergence phase), 36 bigynic colonies were collected, during the first half of May 2015, before worker emergence, from three different sites in the surrounding of Florence (Tuscany, Central Italy). In the same period, foundresses from two different populations in the surroundings of Florence were collected to be used as non-nestmate lures (see below).

For the second experiment (post-emergence phase), 10 colonies in workers phase were collected in the surrounding of Siena at the beginning of July 2016. Non-nestmate workers used as lures were collected on colonies belonging to different populations located in the surrounding of Florence. In both experiments, wasps used as non-nestmates were collected in populations at least 3 km distant from populations where focal experimental colonies were collected in order to minimize the likelihood of high relatedness and prior encounter among tested individuals.

Colonies were brought to the laboratory, and each was transferred to a 15x15x15 cm glass cage provided with *ad libitum* sugar, water, fly maggots and paper as building material. Colonies were maintained under natural light and temperature conditions with additional illumination from Neon lightning with a daily rhythm (L:D 10:14).

In the bigynic nests each foundress was differently marked with enamels on the wings for individual identification. Behavioral observations were carried out before NMR experiments in order to establish the dominant and subordinate individuals for each colony, on the basis of well-

established dominant rank-related behaviors such as ritualized dominance behaviors, egg-laying and low foraging effort (Pardi 1948, Pratte 1989).

General experimental procedure

Each colony was subjected to four NMR trials, which consisted in the simultaneous presentation of two lures carrying NMR cues related to one or both sensory modalities – visual (i.e. wasp head) and chemical (i.e. CHCs) cues – in a concordant (both from a nestmate) or discordant (one from a nestmate and the other from a non-nestmate individual) combination. Lures were presented to colonies in a random order and behavioral response was video-recorded for 1 minute after the first interaction between lure and resident wasps. An aggressive response index was computed as total time spent in aggressive acts (bites and stings) towards the lure (see below “Data Analysis”). Both pre-emergence (n = 36) and post-emergence colonies (n = 10) were tested once with the same protocol.

Lure selection

In the pre-emergence experiment, for each colony two wasp lures (see *Lure preparation*) were selected: *i*) the beta female of the tested nest as nestmate lure, *ii*) a foundress belonging to a different population as non-nestmate lure. Lures were coupled based on a clear different color pattern on the clypeus. Three categories of clypeus pattern were selected: 1 = totally yellow clypeus, 2 = one spot, 3 = two or more spots on the clypeus (Tibbetts & Dale 2004, Tibbetts & Lindsay 2008).

Analogously, also in the post-emergence one, two wasp lures with a different color pattern on clypeus (see above) were selected for each colony: *i*) a worker of the tested nest as nestmate lure, *ii*) a worker belonging to a different population as non-nestmate lure.

Lure preparation

Chemical cues: all wasps selected to be used as a lure were killed by freezing one day before the bioassay. For obtaining CHCs extract, the entire body of each wasp lure was individually placed in a glass vial with 250 µl of an apolar solvent (pentane) for 15 minutes (Dani et al. 1996, Bruschini et al. 2011). After wasp body removal, vials with pentane extracts were left to dry overnight. The following day, before NMR bioassays, extracts were re-suspended in 100 µl of pentane and transferred on pentane-washed filter paper sheets (2.7 x 1 cm). Filter paper sheets were then fixed on an inert support (half cigarette filter paper, ultraslim, Rizla) to obtain lures bearing the sole chemical cues.

Visual cues: after washing the wasp body in pentane, the head of each lure was separated from the rest of the body and was immersed in pentane (1 ml) overnight (Dani et al. 1996,

Baracchi et al. 2015) to totally remove the residual CHCs fraction. The following day, before NMR bioassays, the heads were fixed on entomological pins over the inert support (see above).

Lure presentation

During each of the four NMR trials, couple of lures composed of one out of four possible combination of stimuli obtained by nest-mate and non-nest mate wasps were presented to each colony (Fig. 1a): *i*) visual lures, i.e. washed heads of nestmate and non-nestmate wasps associated with pentane washed filter paper; *ii*) chemical lures, i.e. half cigarette filter paper with filter paper sheet loaded with CHCs extracts of nestmate or non-nestmate; *iii*) concordant lures, bearing together chemical and visual cues of each wasp, recreating the natural coupling of visual and chemical cues where each individual presents his own array of stimuli; *iv*) discordant lures, composed of nest-mate visual cues and non-nestmate chemical cues on one lure and non-nestmate visual cues and nestmate chemical cues on the other, creating an unnatural situation with mixed visual and chemical stimuli to understand whether one stimulus overcomes the other in the NMR.

The procedure for all the experiments consisted of the simultaneous presentation of two stimulus lures (Fig. 1) Following a protocol reported for similar bioassays carried out on the same species (Ortolani et al. 2010; Bruschini et al. 2011; Cappa et al. 2016), we used a 30-cm long stick with a fork at one end. The two different lures (belonging to same combination of stimuli) were mounted on the tips of the fork, 1.5 cm apart, and were randomly placed on the left or right. The fork device was slowly introduced into the colony box while the alpha female (pre-emergence experiment) or the workers (post-emergence experiment) were on the nest and held at a distance of 1 cm from the nest comb for 1 min after the first interaction between the alpha female/workers and the presented lures. In the post-emergence experiment, for each of 10 colonies we performed two set of tests, each one presenting the four couple of lures to a group of different workers (i.e. in total we tested 20 different groups of 5-10 wasps). In post-emergence colonies, immediately before the experiment, foundresses were removed from nest in order to assess only the aggressive response of workers in each of two set of tests.

Colonies were presented with all four combinations of stimuli in a random order at one-hour interval between successive trials. Presentations were video recorded. In each of the two experiments, all the tested colonies were in a similar stage of development (measured as number of cells, larvae, and pupae) to ensure equal motivation in nest defense. Experiments were carried out from 11:00 AM to 15:00 PM, on sunny days. All experiments were performed blindly by a first experimenter and video-recorded by a second experimenter. A total of 144 trials on 36 colonies were carried out in the first experiment (pre-emergence phase) and 80 trials on 20 different groups of workers from 10 colonies in the second experiment (post-emergence phase).

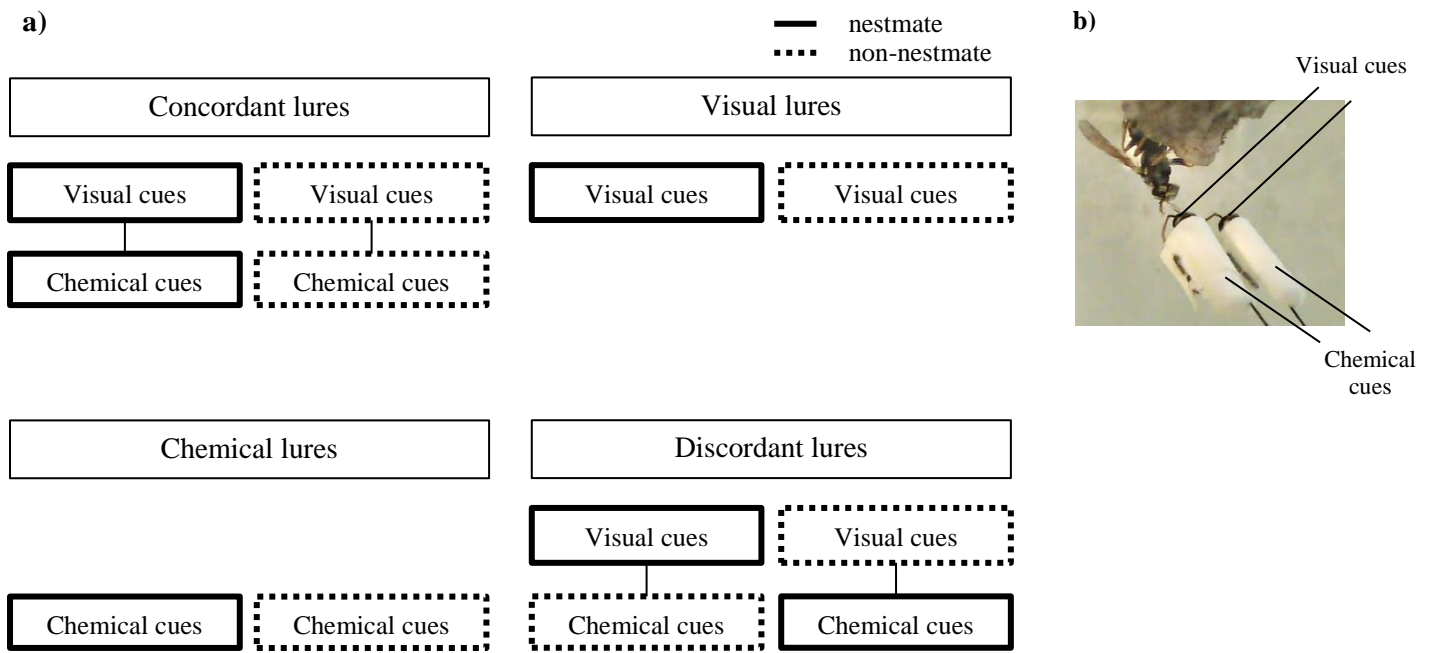


Figure 1 a) Four combinations of stimuli used for b) simultaneous presentation of two lures belonging to same combination.

Data analysis

Video recordings were watched blindly by two observers to avoid biases in counting the interactions between the wasps and the lures during presentations. When approached with lures, behavioral responses of the wasps can be classified into inspective (i.e., workers antennate the presented lure) and aggressive (i.e., workers open their mandibles and attack the presented lure biting and, more rarely, stinging the lure). We considered only the aggressive acts as this is the typical behavioral response that allows to evaluate NMR (Dani et al. 2001). Thus, the time (in seconds) spent by workers in biting and stinging each lure was recorded for statistical analyses.

In order to evaluate NMR we evaluated whether, as expected, aggressive response was greater toward non-nestmate lures than toward nestmate ones, and whether this was depending on the kind of stimuli presented, i.e. visual, chemical and their combination (see above). For each experiment we separately performed a Generalized mixed model, with Poisson distribution and log-link function, followed by post-hoc pairwise comparison with Sequential Sidak correction. We set the aggressive reaction (i.e. time spent in aggressive acts toward the lure) as the dependent variable. Lure category (i.e. nestmate or non-nestmate) and treatment (i.e. only visual, only chemicals, concordant, discordant stimuli) were set as fixed factors. We considered colony id as random factor and trial id as repeated measure (each colony performed 4 trials).

Results

In both experiments aggressive response was significantly influenced by lure category, treatment type and their interaction, but the kind of stimuli allowing efficient NMR was different in the two experiments (Table 1). Considering, in each of the two experiments, the difference in aggressive response between the two lures presented simultaneously, we found that in the pre-emergence experiment, non-nestmate lures were attacked more than nestmate (thus highlighting a proper NMR) when complete concordant stimuli (chemical and visual) and only visual stimuli were presented (Fig. 2a). On the contrary, no significant difference was found in the aggressive response toward nestmate and non-nestmate nor when only chemical stimuli nor when discordant stimuli were presented (Fig. 2a). In the post-emergence period, non-nestmate lures were attacked more than nestmate (thus highlighting a proper NMR) when complete concordant stimuli (chemical and visual) and only chemical stimuli were presented (Fig. 2b). On the contrary, no significant difference was found in the aggressive response toward nestmate and non-nestmate nor when only chemical stimuli nor when discordant stimuli were presented (Fig. 2b).

Source of variance	F-value	df1	df2	Sig.				
					F-value	df1	df2	Sig.
					Early season		Late Season	
Treatment	5.503	3	280	0.001	118.331	3	152	<0.001
Lure	51.025	1	280	<0.001	152.245	1	152	<0.001
Treatment x Lure	9.096	3	280	<0.001	29.921	3	152	<0.001

Table 1 Nestmate recognition is based on visual or chemical cues according to the colonial stage. Results from GLZ show that in both the pre-emergence and post-emergence periods the time spent attacking the lure depends on the lure category (alien vs nestmate), on the focal colony treatment (complete concordant, complete discordant, only chemicals and only visual cues) and by the interaction between these two factors. Sig. = p-value

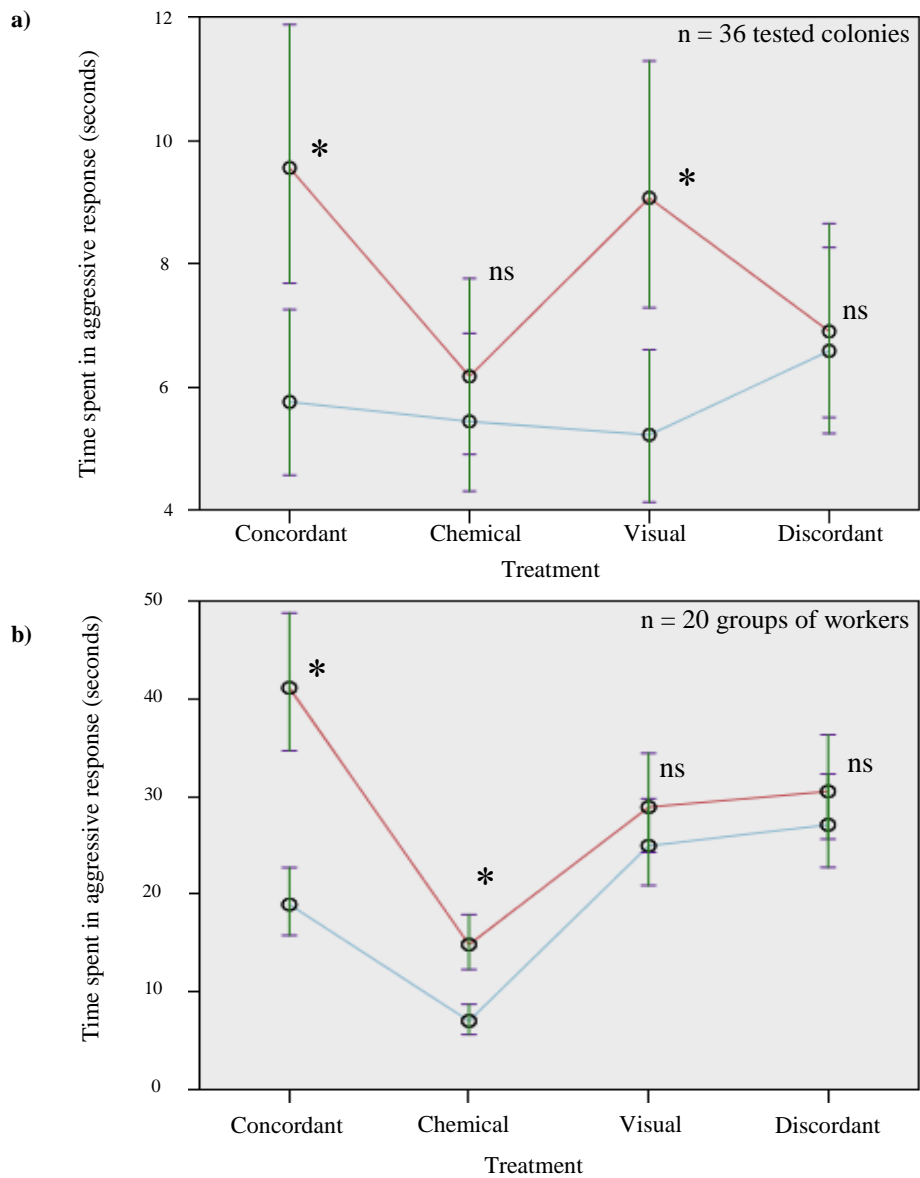


Figure 2 NMR is present when complete concordant lures are presented, and not when complete discordant lures are presented. Visual cues alone allow NMR only in the pre-emergence period (a) while chemical cues alone allowed NMR only in the post-emergence period (b). Circle and bars respectively represent mean and standard error of the mean; Non-nestmate (in red) nestmates (in blue). ns = not significant pairwise comparison, * = significant pairwise comparison ($p < 0.05$).

Discussion

Our results show that the relative importance of nestmate recognition cues of the two sensory modalities, visual and chemical, changes according to colony phase and, therefore, to colony size. In the early phase of the colony cycle, before workers emergence, foundresses favor visual over chemical cues in NMR recognition process. Conversely, in a more advanced colony stage, when a large number of individuals is on the nest, workers rely on the chemical cues rather than on the visual ones to discriminate among nestmates and foreign individuals.

This shift from visual to chemical cues in NMR can be explained by the interplay between the features of the two sensory modalities and the different colonial context across the season, which translates into different reliability of NMR cues in the two different colonial phases. Visual cues are quicker to process than chemical ones, so if colonies are composed only by foundresses and colony size is thus relatively small (ranging from 1 to 10 individuals, usually around 2 – 4; Reeve 1991) is possible that visual cues are adequately informative and these could be used in NMR; on the contrary when colony size rapidly increases up to dozens of wasps, extra information is required and we can speculate that visual cues are not sufficiently informative. Indeed, the variation in the color patterning of the clypeus, the only visual cue so far shown to be perceived and used in intraspecific communication outside the sexual context (reviewed in Cervo et al. 2015), is limited. Actually, in many populations, a significant percentage of wasps show very similar facial pattern (Cervo et al. 2008, Zanette & Field 2009, Green & Field 2011), so that they can be categorized in a few classes (Tibbetts & Lindsay 2008, Cervo et al. 2008, Cervo et al. 2015). This suggests that the reliability of visual cues for NMR rapidly decrease as colony size increases. Moreover, reliability might also decrease because of an intrinsic cognitive difficulty for wasps' brain to remember many visual patterns (Sheehan & Tibbetts 2008, 2011, Baracchi et al. 2016).

Previous studies seem to suggest that the clypeal color patterning, which is genetically and environmentally determined (Tibbetts & Curtis 2007, Tibbetts 2010, Green et al. 2012), could be less variable within the colony in post-emergence than in pre-emergence phase and thus it could be used in late colonies as colonial label. However, in crowded post-emergence colonies many different kinds of facial patterns are present (pers. obs.) and this could make less effective and reliable the visual cues-based NMR. Moreover, in early colonies, the presence on nest of few individuals that show different clypeal patterns might favor the use of visual cues in NMR reducing the cognitive effort of memorization. (Tibbetts & Dale 2007, Baracchi et al. 2016). However, further studies are needed to evaluate if actually the visual patterning is so variable among workers of the same colony to invalidate its use as colonial label in NMR context. It will

also be interesting to assess if the intra-colony variability of clypeal coloration pattern is less than or greater than the variability among members of different colonies to evaluate the reliability of visual cues in the two different colony phases.

On the contrary, chemical cues might be more reliable after the emergence of workers than in pre-emergence phase. Despite a proper comparison of reliability of CHCs as NMR cues in different phases of colony life has never been done, it is conceivable that early season colonies (and thus pre-emergence ones) have a less marked colonial chemical signature than advanced stage colonies. This is suggested by *i*) the finding that foundresses show very similar CHCs profiles at colony foundation that rapidly diverge (immediately before the emergence of workers), acquiring a differentiation based on colonial membership (Sledge et al. 2001); *ii*) the more homogeneous composition of advanced colonies, both in terms of physiology (for example in terms of fertility) and relatedness, compared to pre-emergence colonies. Indeed, few weeks after workers emergence, the colony is composed by the dozens of sister workers, which thus share the genotype and many physiological feature (above all, they are almost all unfertile or poorly fertile) (Queller et al. 2000). On the contrary, small pre-emergence colonies show a higher heterogeneity, with wider variation in the physiological status of foundresses (Pardi 1946, 1948, Röseler et al. 1980, Röseler 1991) and relatedness (Queller et al. 2000, Leadbeater et al. 2011), all factors that are known to affect CHCs individual profile (Bonavita-Cougourdan et al. 1991, Sledge et al. 2001, Dapporto et al. 2004, Dapporto et al. 2005). As the colonial chemical signature is the product of a template shared by all individuals thanks to social interactions (contacts, trophallaxis) and through the nest material (Signorotti et al. 2015), it is thus likely that the more homogenous conditions of late-season colonies allow the production of a more marked and reliable colonial chemical signature, while in pre-emergence colonies individual level heterogeneity might somehow reduce the intercolonial differences in the chemical profile.

The finding that in each colonial phase visual or chemical cues, respectively, were not sufficient to allow NMR does not mean that they had no influence at all in the decisional process of wasps. Indeed, when both visual and chemical stimuli were coupled on the sample lure in a discordant combination (thus with both nestmate and non-nestmate cues on the same lure) wasps were not able to tell distinguish nestmates from non-nestmates, even if the important set of cues (i.e. visual in pre-emergence experiment, chemical in the post-emergence experiment) was still present. This suggests that the discordance in provided cues weakened the NMR process. Interestingly, this suggest a possible cross-modality sensory integration.

Taken together, our results open the possibility that cues used in NMR by *P. dominula* could dynamically change through colony cycle. However further studies must be conducted to better identify the factors that determinate this change in NMR response according to colony phase.

We highlight two main possible limitations of our study. First, our study compared two very different periods, to cover the wide variation in context that colonies experience. However, this means that, to respect the natural conditions, focal wasps subjected to NMR trials were foundresses in the first experiment and workers in the second. While we can't discard the hypothesis that caste-differences exist, especially in the information-processing ability (Injaian & Tibbetts 2014), we believe that this is a less likely, even if not impossible, situation. Indeed, *P. dominula* show a very weak castal differentiation, which is mainly behavioral rather than physiological (Pardi 1948, Reeve 1991).

Second, in pre-emergence colonies we recorded the response of the only individual present on the nest (colonies were founded by two females, one of which became the nestmate lure) while in post-emergence experiment the response of many workers present was recorded. While analytically this does not represent a problem, as comparisons were internal to colony phase and colony identity, one might speculate that group dynamics influence more the use of one sensory modality than the other. Future studies should thus investigate the use of visual and chemical cues for nestmate recognition focusing on the same individual (thus having the same phenotype, i.e. foundress or worker) through their entire life-cycle in contexts where reliability of cues differ (i.e. the same queen before and after worker emergence, or the same wasps in nest by altering experimentally phenotypic variation in NMR cues).

We believe our results provide several interesting insights, both at the taxon-specific level (*Polistes* paper wasps) and at a wider perspective. First, at the taxon-specific level, we demonstrated that visual cues alone can allow NMR in specific context, which is this within eusociality evolved. In this case this type of social recognition can be considered familiar recognition. This is the first such finding for a Polistine wasps, as so far NMR based on visual cues has been shown only for hover wasps (Baracchi et al. 2015). This finding indeed open interesting perspective on the highly debated topic of the use of visual communication in *Polistes* paper wasps, in which the absence, presence and different level of the use of visual cues are proved in different species and populations for a wide range of social recognition processes, from familiar recognition to gender recognition (reviewed in Cervo et al. 2015). Under this perspective, our results add experimental evidence for the importance of visual cues in this species, thus making things even more complex. Moreover, we unexpectedly found that chemical cues alone are not sufficient in pre-emergence to allow NMR, which suggest that the long-lasting tenet that

NMR in social insects is governed by chemicals not necessarily hold true for all species in all contexts.

Under a wider perspective, our results also suggest two important concepts. First, we argue that NMR can take the shape of familiar recognition in small groups and of NMR in large societies. It is thinkable that within animal groups that shift from being small associations to large societies, group members first learn to recognize individual by familiarity (and possibly by individual recognition, also suggested for *P. fuscatus* and other social insects, Tibbetts 2002, d’Ettorre & Heinze 2005) and then, when colonies become large, shift to NMR. In the latter individual are recognized as nestmate if they bear the specific colonial label (Gamboa et al. 1986, Dani 2006, van Zweden & d’Ettorre 2010). This is a drastically different process from the one that occurs in the perennial large societies of many ants, termite and bees, in which colony foundation by swarming or colony fission prevent the “small society” phase, thus precluding the possibility of a familiar recognition.

More generally, our results suggest an underestimated plasticity in the mechanisms of social recognition within the same species across different contexts. We believe this might have a profound impact as it highlights that the external validity of animal communication results might be really limited if performed in studies focusing on a single and/or specific context (or life stage or phenotype). Unfortunately, there is a dramatic lack of replication studies in animal (and especially insects) communication studies. Given its biological features and the easiness of manipulation, *P. dominula* will certainly represent a fruitful model to assess these topics in the future.

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Authors contribution

AC, RC planned the experiment, AC, FC, IP, LP performed the experiment and analyzed behavioral data, AC analyzed data, AC wrote the paper, RC provided funds, all authors read, commented and finally agreed on the MS.

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4. Vibrational co-evolution: exaggerated mimicry of the host's signal by a wasp social parasite

Rachele Nieri^{1,2§*, x}, Irene Pepiciello^{1*}, Alessandro Cini^{1,#}, Valerio Mazzoni² and Rita Cervo¹

1 Dipartimento di Biologia, Università di Firenze, Via Madonna del Piano 6, 50019 Sesto Fiorentino (FI), Italy.

2 Dept. of Sustainable Ecosystems & Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, via Mach 1, I-38010 San Michele all'Adige (TN), Italy.

#Current address: Centre for Biodiversity and Environment Research, University College London, Gower Street, London WC1E 6BT

x Current address: Center Agriculture Food Environment, University of Trento, via Mach 1, 38010, San Michele all'Adige (TN), Italy

§ corresponding author: Rachele Nieri, mail: rac.nieri@gmail.com; * equally contributing authors

Abstract

The ability of insect social parasites to exploit host communication systems and break host nest defense to enter and obtain integration within the host colony, has been deeply investigated. The parasite integration can occur by mimicking or exaggerating host chemical, visual and mechanical signals, but, until now, it has been assumed that parasite strategies are mainly governed by chemicals. Here, we used the paper wasp *Polistes dominula* – *Polistes sulcifer* pair, a well-known social insects host-parasite system, as a model to investigate if the vibrations produced by parasite match or exaggerate those produced by the host. In *P. dominula*, indeed, previous papers described vibrations produced by a well-defined abdominal lateral oscillation called abdominal wagging (AbW), but such vibrations were never compared to the parasite ones. By using a laser vibrometer we recorded the vibrations produced by host queen on both non-usurped and usurped nests. On usurped colonies, we also recorded the parasite vibrations generated by its peculiar oscillatory abdominal vertical movement (i.e. abdominal drumming, AbD). Results show that both species produce surface-borne vibrations consisting of groups of repeated broad-band pulses but the vibrations produced by the parasite can be discriminated by the host queens mainly for the longer and more intense events. At the same time host queens on usurped nests performed shorter and less intense AbW than those produced by host queens and recorded on queen-right colonies. Our results support the hypothesis that parasite vibrations, working in synergy with ritualized dominance behavior, could be used by host and parasite as a signal towards both larvae and adult to impose and maintain the dominant role on the nest.

Keywords: paper wasp, *Polistes*, abdominal wagging, social parasite, laser vibrometer, biotremology.

Introduction

Insect colonies represent the target of many social parasites, i.e. species that, instead of investing in brood care, exploit the eusocial structure of host species to produce their own offspring (Wilson 1971). As the parasitic strategy can impose severe fitness costs to the host, by hampering its reproduction output, natural selection promoted the evolution of defensive mechanisms in host species. In turn, social parasites developed a variety of deceptive strategies to minimize the ability of the host to detect and react to their intrusion. Within this evolutionary arms race, it has been widely demonstrated that a key strategy to ensure host altruistic services is to intercept and use the host communication code to manipulated host's society (Wilson 1971, Dawkins and Krebs 1979, Dettner & Liepert 1994), e.g. larvae of social parasite butterflies of genus *Maculinea* mimic and exaggerate chemical and mechanical signals of host to be brought into the nest and be cared by *Myrmica* workers (Akino et al. 1999, Barbero et al. 2009).

In the most extreme form of social parasitism, i.e. obligate insect social parasites, the worker caste has been lost and thus the parasites have to completely rely on the host worker force to rear their own brood (Wilson, 1971). They must necessarily overcome the host nestmate recognition system to successfully enter the nest and then they must integrate themselves in the host colony to be accepted in the social group (Nash & Boomsma 2008, Bagnères & Lorenzi 2010).

Obligate social parasites of the primitively eusocial *Polistes* wasps represent a well-known model of social parasitism (Cervo 2006; Lorenzi 2006) and provide a good opportunity to study how parasites intercept and exploit host communication system to infiltrate into its social structure. Their ability to exploit the chemical host communication systems has been widely investigated. It ranges from distracting host defenders by emitting a venom volatile bouquet enriched in some compounds probably involved in increasing intra-colonial aggressiveness (Bruschini & Cervo 2011) to “breaking into the colony fortress” by becoming undetectable. For the latter strategy parasite can lower the recognition cues (Lorenzi & Bagnères 2002, Lorenzi et al. 2004, Cervo et al. 2008) and/or match the host colony chemical signature to acquiring the chemical profile of the specific usurped colony and even that of the former dominant individual (i.e. cuticular hydrocarbons are known to play a crucial role in nest-mate recognition) (Bagnères et al. 1996; Turillazzi et al. 2000; Sledge et al. 2001; Lorenzi and Bagnères 2002; Dapporto et al. 2004; Lorenzi et al. 2004; Lorenzi 2006).

Moreover, in the last years, a body of work started to elucidate the role of the conspicuous visual facial patterning of the parasites, which seems to exaggerate the visual signal typical of the host species (Ortolani et al. 2010; Green & Field 2011; Cini et al. 2011a; Cini et al. 2015; Cervo et al. 2015). All this evidence reported so far suggests that the selection pressure driven by the

coevolutionary arms race is so strong that social parasites are pushed toward the interception and exploitation of all important host communication channels. Under this perspective, it is surprising that, until now, no studies examined the possibility that the *Polistes* social parasites employ sensory deception strategies also within the vibrational communication channel. Indeed, vibrational communication is widespread in *Polistes* wasps (Jeanne 2009, Hunt & Richard 2013) and oscillatory behaviors potentially producing substrate-borne vibrations have been described for at least 18 species of genus *Polistes* (Brennan 2007). These behaviors consist in oscillatory movements of entire body or its parts, i.e. abdomen or antennae, which in many cases hit the nest producing substrate-borne vibrations. The function of these vibrations is not clearly understood; however, they are considered to be signals directed both to the larvae growing inside the comb cells and to the adults living in the colony (Jeanne 2009). It is believed that these putative signals play a role in the regulation of many crucial aspects of social organization within the colony. Studies carried out on different *Polistes* species reported that such vibrations are produced *i*) more by dominant and breeding females than subordinates and workers, *ii*) especially during inspection and feeding larvae. Moreover, oscillatory behaviors are performed *iii*) during aggressive as well as ritualized encounters between dominant and subordinate individuals, and *iv*) are more common during the pre-emergence phase, rather than later in the season, when hierarchies need to be established and larvae present in the comb will developed in workers (Gamboa & Dew 1981, Brillet et al. 1999, Suryanarayanan et al. 2011, Mignini & Lorenzi 2015). Based on this, Jeanne (2009) suggested that the various forms of vibration movements performed by dominant individuals both during feeding and non-feeding contexts represent a continuum of the same phenomenon that acts, by means of a series of biochemical events, on the reproductive physiology both on larvae and subordinate adults: this highlights the crucial role of such mechanical signals on social organization of *Polistes* wasps. Such hypothesis has been partially confirmed on larvae in an American species, *Polistes fuscatus*, through a playback experiments on developing larvae (Suryanarayanan et al. 2011, Jandt et al. 2017) by showing that the vibrations produced by antennae would act as a modulator of nourishment affecting larval growing rates and development of workers-specific traits.

Under this scenario of multiple and crucial roles of such vibrations affecting social life in a *Polistes* colony, we can expect that *Polistes* social parasites evolved strategies to intercept and exploit the host vibrational signals to integrate and secure fitness on host colonies as they do for chemical and visual communication modalities (Turillazzi et al. 2000, Sledge et al. 2001, Ortolani et al. 2010).

In *Polistes dominula*, the only host species of obligate social parasite *Polistes sulcifer* (Cervo 2006), previous papers described a well-defined abdominal lateral oscillation called abdominal wagging (AbW) (Brillet et al. 1999, Brennan 2007); such behavior transmits vibrations to the nest that have been recorded by an accelerometer and analyzed in detail (Brennan 2007). Very intriguing, the females of *Polistes sulcifer* perform a vigorous movement of the abdomen perpendicular to the nest, which is so intense to produce an audible sound and called abdominal drumming (AbD) (Cervo 1990; Cervo 2006). Likely this behavior is responsible for substrate-borne vibrations at the same manner that host foundress AbW does. Although, the behavior itself and if it transmits vibrations into the nest remain unknown. If so, it is possible that the parasite exploits also the vibrational system of the host to communicate its dominant and central role in the usurped colonies, both to host adults and larvae.

Here we investigate if, as we expect, the social parasite produces vibrational signals by means of AbD when it becomes a member of the host colony and acquires the higher rank position in the colony by eliminating or submitting the former dominant queen (Turillazzi et al. 1990, Cervo 1990).

To describe the physical attributes and compare the substrate-borne vibrations produced by the two evident oscillatory behaviors of host and social parasites females on the nest, we used a “last-generation” recording equipment (i.e. laser Doppler vibrometer). The laser enabled us to record the wasp induced vibrations without altering them by adding the instrument load to the substrate, as it has been done in previous studies (Brennan 2007).

We aim to describe the physical attributes of the abdominal vibrations produced by the parasite and its host. We then use these attributes to test the following hypotheses: 1) parasite AbD and host AbW have a similar structure; 2) parasite vibrations exaggerated some parameters of host signal; 3) the presence of parasite (dominant female) on nests affect vibrational signals emitted by host females.

Materials and methods

Host and parasite colony cycle

P. dominula colonies are founded in spring by one or more mated foundresses; in the latter case, soon after foundation, a linear hierarchy is established by agonistic interactions and later it is maintained by means of ritualized dominance behaviors (Pardi 1996). One foundress becomes the dominant female and lays most of the eggs (more than 90%, Queller et al. 2000) while the other females become subordinates and perform all the colonial works. At the end of May, the worker-phase starts with the emergence of the first workers. Workers help until the end

of the season rearing additional brood. Reproductive individuals, males and females, emerge only later in the season, from the end of July (Reeve 1991).

P. sulcifer parasite females fight with *P. dominula* foundresses to usurp their nest prior the emergence of workers (Turillazzi et al. 1990; Cervo and Turillazzi 1996; Ortolani et al. 2008). During these violent fights the dominant host female is killed or it is thrown out of the nest by the parasite; sometimes, after usurpation, former dominant female returns to the nest and assumes a subordinate role (Turillazzi et al. 1990). As soon as usurpation is successful, to acquire the nest chemical signature, the parasite chemically integrates itself in the host colony performing an intense nest stroking behavior and takes the role of the higher ranked female by mimicking its chemical profile (Turillazzi et al. 1990, Sledge et al. 20010, Dapporto et al. 2004, Cini et al. 2011b). Moreover, to establish and maintain the dominant position, once on host nest, the parasite female implements and exaggerates physical dominance towards the host females. Even if the host reproductive activity is not completely suppressed (Cini et al. 2014), the parasite lays its eggs in the nest and relies exclusively on the host worker force to rear its brood which are represented by only reproductive individuals (Cervo 2006).

Animal collection, laboratory rearing and usurpation trials

To record the vibrations produced during AbW by foundresses present in non-usurped colonies without interference of additional individuals, we used solitary foundations of *P. dominula* (i.e. a single foundress starts a colony at the beginning of spring) before workers emergence. Nine solitary colonies, that had 35.09 ± 12.36 (mean \pm SD) cells, were collected during spring 2014 in various Italian sites.

The parasite prefers to usurp pre-workers associative colonies (Cervo & Turillazzi 1996). Thus, to record vibrations produced by the parasite and by host foundresses after nest usurpation, 14 pre-workers solitary (n = 5) and associative (n = 9) colonies were collected in spring 2015. Colonies had 56.27 ± 15.34 (mean \pm SD) cells at the time of collection.

At the collection all colonies were full of immature brood (5.55 ± 7.09 (mean \pm SD) and 9.71 ± 5.59 eggs, 12.73 ± 5.26 and 24.43 ± 15.41 larvae and 4.82 ± 4.85 and 8.14 ± 4.04 pupae in non-usurped and usurped colonies respectively). All colonies were transferred to laboratory where they were maintained in plastic cages (15x25x15 cm) for the entire experimental period. Water, sugar and *Galleria melonella* larvae and fly maggots were provided *ad libitum* to each colony. All cages were kept at 25 ± 2 °C and natural daylight cycle. When multiple foundresses were present, each individual was marked with different colors on thorax and/or wings.

P. sulcifer females were collected in 2015 at the end of the overwintering phase (May) in central Italy. Once in laboratory, they were kept under overwintering conditions (7 °C) until

activation. Parasites were activated at room temperature for 7 days, following Ortolani et al. (2008) protocol. After activation, at the end of May – beginning of June (when the usurpations occur in the wild, Cervo & Turillazzi 1996), a random host colony to usurp was offered to each parasite by introducing a parasite inside a host colony cage and leaving the parasite free to approach and conquer the host colony (Turillazzi et al. 1990; Cini et al. 2011a). Successfully usurped colonies (n = 11) were maintained in laboratory condition as previously described for the host ones.

Recording trials

Behavioral observations were conducted at a temperature range of 25 – 30 °C and in the central hours of the day (11:00 – 16:00), when wasps are more active on the colony. Individual's behaviors and abdominal vibrations were recorded simultaneously by means of a video camera (HDC-TM700, Panasonic Corporation of North America) and a laser vibrometer respectively (Ometron VQ-500-D-V, Brüel and Kjær Sound & Vibration A/S, Nærum, Denmark; PDV 100, Polytec, Germany). Prior to mount the nest in the cage, a reflective sticker was attached on its back surface and used to focus and maximize the sensitivity of the laser beam. The substrate-borne vibrations were digitized with 48 kHz sample rate, 16-bit depth, and stored directly onto a hard drive through a multichannel LAN-XI data acquisition device (Brüel and Kjær Sound & Vibration A/S, Nærum, Denmark). All trials were carried out during the second half of May and the first days of June for both years, which corresponds to the natural period of the host colony cycle when the first workers are ready to emerge or just emerged and the parasite usurpations occur.

Each not-usurped colony was recorded in random order twice during pre-emergence phase for 15 minutes. At the recording time, in the nest were present 12.00 ± 4.36 (mean \pm SD) larvae.

Usurped colonies were recorded from three days after usurpation when chemical integration of parasite females in host colonies is complete (Turillazzi et al. 2000). Since AbD is less frequent compared to AbW (Cervo 1990), to record a comparable number of body oscillation of the parasite and the host, each usurped colony was recorded from one to three times for a minimum of 15 minutes. During the recording of the usurped nests 1.21 ± 0.70 (mean \pm SD) host foundresses, 2.43 ± 1.50 workers and 21.82 ± 15 larvae were present.

Terminology and data analysis

Described vibrations were named as the behavior that produced them: the vibrations produced by host oscillatory movement were called abdominal wagging (AbW) (Brennan 2007) and those generated by vertical oscillations of parasite abdomen were called abdominal drumming (AbD) (Cervo 1990, Cervo 2006). AbW produced by *P. dominula* in usurped colonies were called

AbW_U, to distinguish them from those produced in non-usurped colonies (i.e. AbW). We define an ‘event’ as the substrate-borne vibrations recorded with the laser vibrometer while the wasp was oscillating or beating the abdomen on the nest surface (Brennan 2007). A ‘pulse’ was defined as a physically homogeneous sound, composed of a brief succession of sine waves (Alexander 1967) (Fig. 1).

Vibrations recorded by the laser vibrometer and produced during AbW or AbD were analyzed from selected colonies. Only colonies in which the focal individual performed more than two signals during the trial were analyzed. Among analyzed individuals, the three following categories of signal were defined according to the signaling individual and colony’s usurpation status: AbW signals produced by *P. dominula* foundress on non-usurped colonies (colonies n = 5; signals analyzed per individual: min = 13 and max = 49), AbD signals performed by parasites (colonies n = 7; signals analyzed per individual min = 5 and max = 30), and AbW_U as AbW performed by *P. dominula* foundresses in usurped colonies (n colonies = 6; signals analyzed per individual min = 3 and max = 28).

Spectral and temporal parameters of recorded vibrations were analyzed with Raven Pro 1.4 (The Cornell Lab of Ornithology, Ithaca, NY) using Fast Fourier Transform (FFT) type Blackman with window length of 256 samples and 50% overlap. To characterize the temporal properties of AbW and AbD the following parameters were measured based on the recorded oscillogram for each event: duration (i.e. the time in seconds between the onset of the first pulse and the end of the last one), number of pulses composing it, and rate of pulses, as the ratio between number of pulses and the event duration. Besides temporal features that were described also in previous studies for AbW (Brillet 1999, Brennan 2007), spectral parameters of the vibrations induced by wasps’ body oscillation movements into the nest were measured from the spectrogram. The laser Doppler vibrometer enabled us to record the whole frequency profile of vibrations propagating into the nest; the dominant frequency (i.e. the frequency with the highest value of energy) of each event and pulse composing it was measured.

The intensity of substrate-borne vibrations depends on the distance of the individual producing them from the detection site of recording (i.e. laser beam focused on the nest), which varies within and between different colonies. For this reason, to compare the intensity of the three vibrations categories (i.e. AbW, AbW_U, and AbD) the ratio between the relative amplitude (RMS) (Chariff et al. 2010) of each event and the vibrations produced by walking at the same distance from the laser beam was measured for each individual.

A discriminant analysis was used to determine whether vibrational events could be distinguished between species and conditions (host in usurped colonies, host in non-usurped

colonies and parasite). To verify if there was correlation between measured parameters we performed Pearson's correlation test. Since, in both parasite and host species, the dominant frequency of pulses is highly correlated with the dominant frequency of events ($p < 0.001$), and duration of events is correlated with the number of pulses present in each event ($p < 0.001$) and the rate of pulses ($p < 0.005$), for the comparison among the specie and the conditions we tested only the following parameters: dominant frequency, duration and intensity of each event. To verify if dominant frequency of AbW_U and AbD vibrations do not differ from dominant frequency of AbW we performed a Generalized Linear Mixed Model (GLMM) with multivariate normal random effects using Penalized Quasi-Likelihood (PQL) method, by considering the vibrations category as fixed factor and individual as random factor. Since the 72% of events has 32 Hz as dominant frequency (Table 3, Fig. 3), before to run the model, we binarize data by assigning the value 0 to events that have 32 Hz as dominant frequency and the value 1 to events that have a dominance frequency greater than 32 Hz.

To compare the duration and intensity (response variable) of vibrational events through AbW, AbW_U, and AbD (considered as fixed factors) two linear mixed models were performed (Type III sums of squares). Individual was included in the model as random factors.

Results

Host and parasite substrate-borne vibrations description and similarities (Table 1-3; Fig. 1-3)

Concurrent analysis of the video and laser vibrometer recordings revealed that the abdominal wagging behavior performed by the *P. dominula* foundress on the nest produced a vibrational event (AbW) detectable by the laser vibrometer and distinguishable by its oscillogram and spectrogram (Fig. 1). Also the parasite produced distinguishable vibrational events (AbD) when performing abdominal drumming (Fig. 2). Both vibrational events, AbW and AbD, consisted of a repetition of broad-band pulses.

Usually, AbW events lasted less than one second, but their duration was particularly variable ranging from a minimum of 0.04 seconds to a maximum of 2.61 seconds. The AbW dominant frequency was on average 39.92 ± 19.06 (mean \pm SD) Hz and 48.10 ± 26.60 Hz (Table 1) in usurped and non-usurped colonies respectively. Each event of AbW was composed of 6.70 ± 3.79 (mean \pm SD) pulses and 7.27 ± 4.26 pulses in usurped ad non-usurped colonies respectively, and the number of pulses per second was variable from a minimum of 2 to a maximum of 31 (Table 1, Fig. 1).

Typically, the AbD produced by parasites lasted 1.51 ± 1.05 (mean \pm SD) seconds and on average consisted of 22.80 ± 15.60 (mean \pm SD) pulses. Pulses were repeated at the rate of 15.69 ± 3.81 (mean \pm SD) pulses per second, but the rate was particularly variable ranging from a minimum of 6 to a maximum of 49 pulses per seconds. As in AbW, also in parasite vibrations most of the energy produced by AbD was concentrated below 100 Hz (Table 1, Fig. 2).

Type	<i>P. dominula</i>		<i>P. sulcifer</i>
	AbW	AbW_U	AbD
N	165	97	160
Event			
Duration	0.61 ± 0.41	0.48 ± 0.34	1.51 ± 1.05
Domfreq	48.10 ± 26.59	39.92 ± 19.06	45.80 ± 26.03
N pulses	7.27 ± 4.26	6.70 ± 3.79	22.80 ± 15.60
Pulses rate	14.01 ± 6.47	16.04 ± 5.61	15.69 ± 3.81
Intensity	2.03 ± 0.90	2.06 ± 0.90	2.69 ± 1.57
Pulse			
Duration	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Domfreq	48.57 ± 19.09	38.93 ± 16.14	51.31 ± 20.19

Table 1 Spectral and temporal parameters of host AbW and parasite AbD. AbW = AbW in not usurped colonies; AbW_U = AbW in usurped colonies; AbD = abdominal drumming. All values are given as mean \pm SD. N = number of events analyzed; Duration is given in seconds; Domfreq = dominant frequency is given in Hz; Pulse rate = ratio between the duration of the event and the number of pulses composing it; Intensity is a dimensionless sample unit.

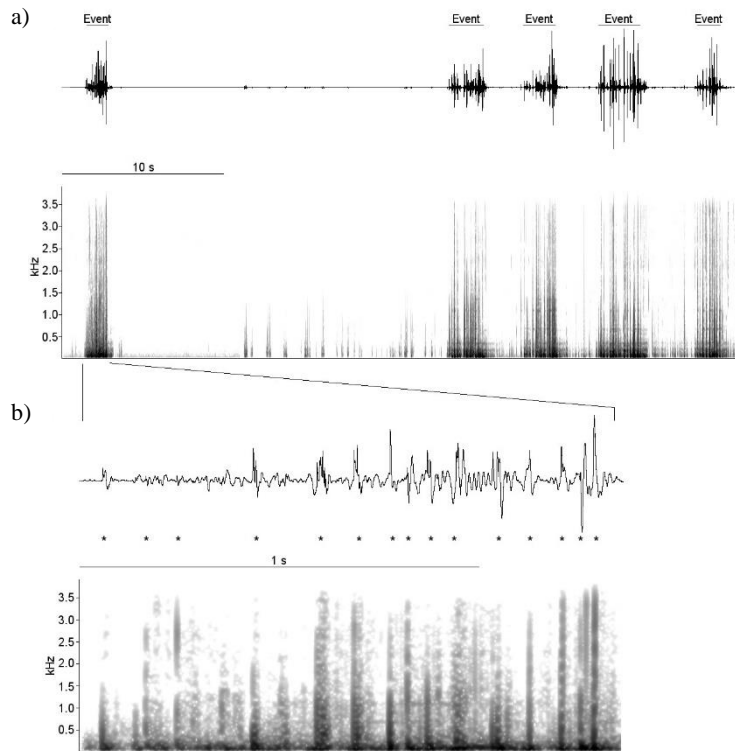


Figure 1 a) Oscillogram (above) and spectrogram (below) of five subsequent events of AbW performed by a *P. dominula* foundress on the nest. In b) the detail of an event, asterisks indicate the pulses composing the AbW.

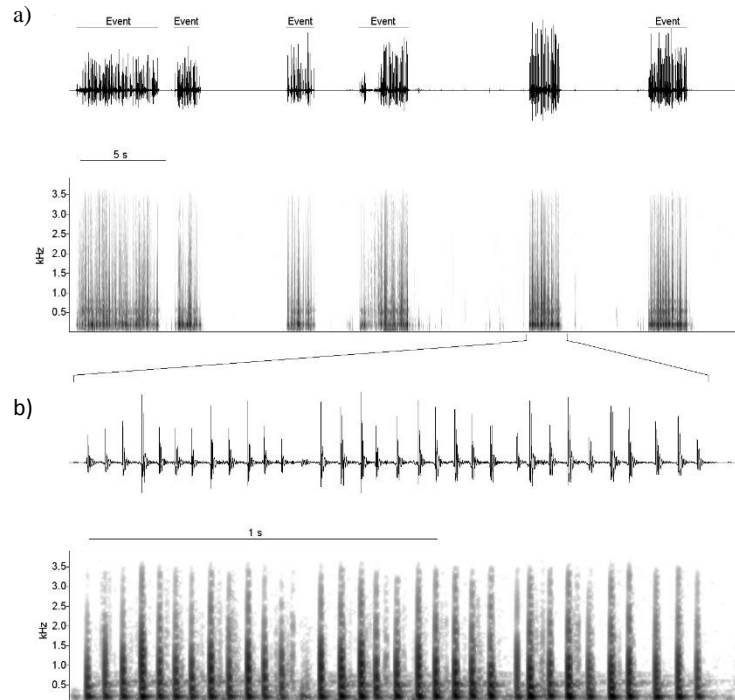


Figure 2 a) Oscillogram (above) and spectrogram (below) of six events of AbD produced by a *P. sulcifer* female on a usurped nest. In b) the detail of an event.

In addition to the analogous structure, the dominant frequency of events recorded on usurped nests did not differ from dominant frequency of AbW produced on non-usurped nests and the respectively 98%, 98% and 96% of dominant frequency of AbW, AbW_U and AbD fall between 32 and 96 Hz (Table 2-3, Fig. 3).

	Value	Std. Error	DF	t-value	sig.
intercept	-2,887	1	404	-1,977	0,049
AbW_U	0,543	1,814	1	0,300	0,815
AbD	2,195	1,774	1,000	1,238	0,433

Table 2 Dominant frequency of vibrational events. Generalized Linear Mixed Model was performed to assess the difference of vibrations recorded on usurped nests from vibrations recorded on queen-right colonies. AbW_U = AbW in usurped colonies; AbD = abdominal drumming; sig. = p-value

Frequency (Hz)		AbW	AbW_U	AbD	Total
32	Count	115a,b	80b	109a	304
	% within event domfreq	37,8%	26,3%	35,9%	100,0%
	% within AbW/AbW_U/AbD	69,7%	82,5%	68,1%	72,0%
64	Count	20a	11a	41b	72
	% within event domfreq	27,8%	15,3%	56,9%	100,0%
	% within AbW/AbW_U/AbD	12,1%	11,3%	25,6%	17,1%
96	Count	27a	5b	7b	39
	% within event domfreq	69,2%	12,8%	17,9%	100,0%
	% within AbW/AbW_U/AbD	16,4%	5,2%	4,4%	9,2%
128	Count	3a	1a	0a	4
	% within event domfreq	75%	25%	0%	100%
	% within AbW/AbW_U/AbD	1,8%	1%	0%	0,9%
160	Count	0a	0a	1a	1
	% within event domfreq	0%	0%	100%	100%
	% within AbW/AbW_U/AbD	0%	0%	0,6%	0,2%
192	Count	0a	0a	2a	2
	% within event domfreq	0%	0%	100%	100%
	% within AbW/AbW_U/AbD	0%	0%	1,2%	0,5%
Total	Count	165	97	160	422
	% within event domfreq	39,1%	23%	37,9%	100%
	% within AbW/AbW_U/AbD	100%	100%	100%	100%

Table 3 Distribution of dominant frequency of vibrational events. AbW = AbW in not usurped colonies; AbW_U = AbW in usurped colonies; AbD = abdominal drumming. domfreq = dominant frequency (given in Hz)

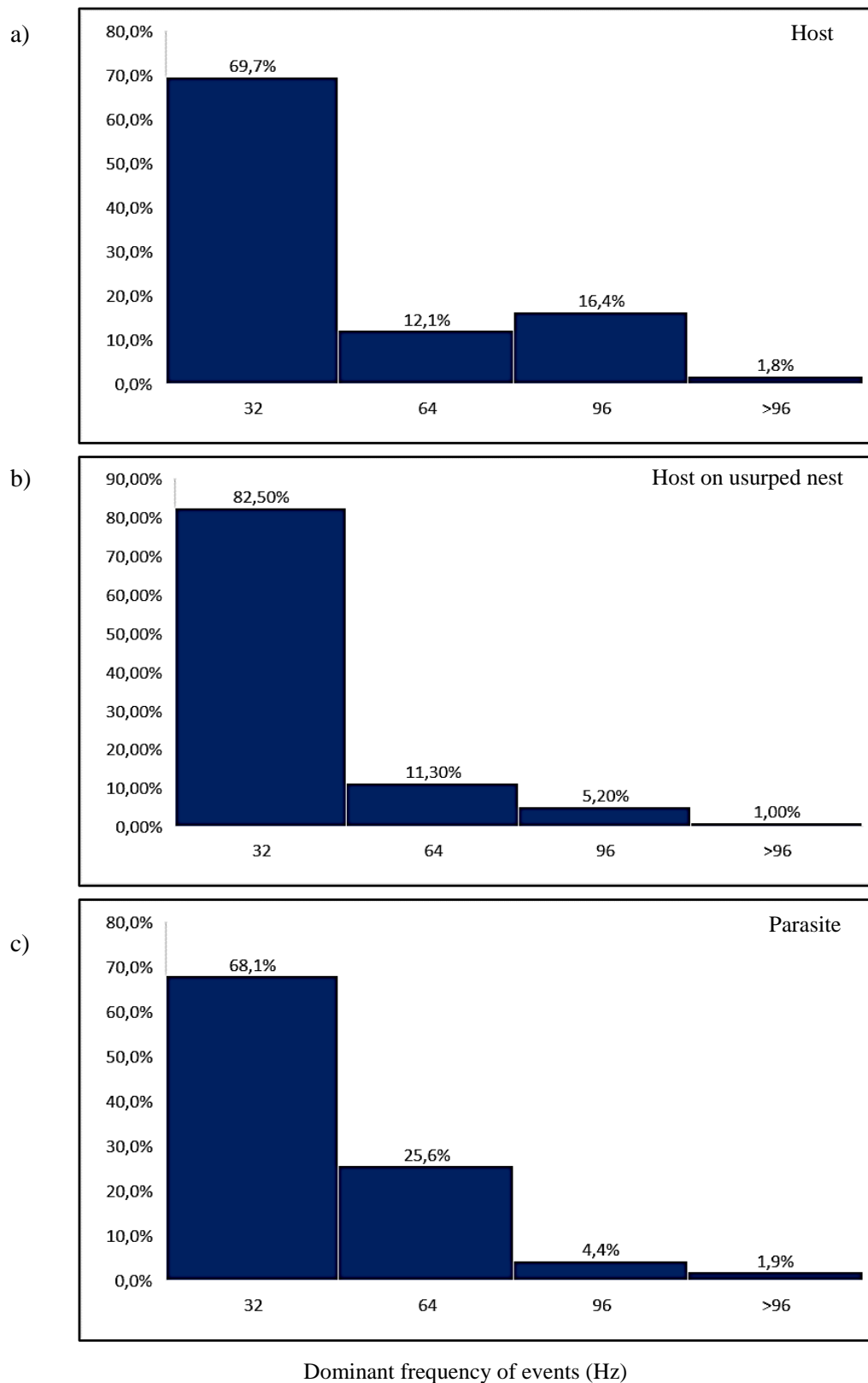


Figure 3 Distribution of dominant frequency (in Hz) of vibrational event in a) host on non-usurped nest, b) host on usurped nest, c) parasite

Comparison between host and parasite vibrations (Table 4, Fig. 4-5)

The discriminant analysis revealed that the intensity of events and their duration have the main role in differentiating AbD and AbW performed in different contexts. The correct classification was 77%. The most error of classification occurs in parasites which are mis-predicted as host (Fig. 4).

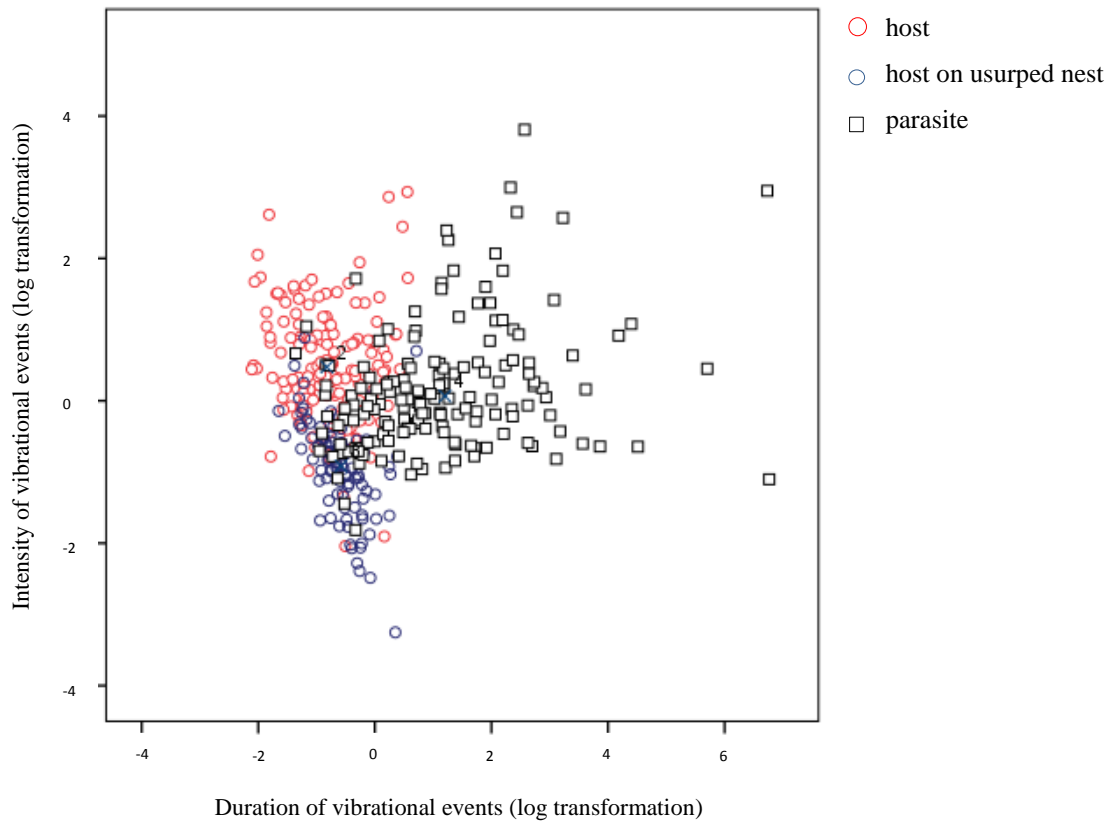


Figure 4 Discriminant analysis revealed that the intensity and duration of vibrational events have a main role in differentiating parasite and host vibrations. Distribution of host and parasite vibrational events along two axes that report log-transformed duration and intensity of vibrational events.

The vibrational events produced by the parasite during AbD were longer and more intense compared to AbW produced by foundresses in the two contexts (i.e. not usurped and usurped colonies). Moreover, the AbW events performed by foundresses in usurped colonies were shorter and lower in intensity compared to AbW produced in absence of the parasite in not usurped colonies (Linear mixed model Type III analysis: duration of event $F = 96,330$, $p < 0,001$, intensity of event $F = 76,340$, $p < 0,001$) (Table 4)

			Intensity			Duration		
			Mean Diff.	SE	sig	Mean Diff.	SE	sig
Host	x	Host on usurped nest	0.443	0.057	<0.001	0.235	0.09	<0.001
		Parasite	-0.26	0.049	<0.001	-0.876	0.078	<0.001
Host on usurped nest	x	Host	-0.443	0.057	<0.001	-0.235	0.09	<0.001
		Parasite	-0.703	0.057	<0.001	-1.111	0.091	<0.001
Parasite	x	Host	0.26	0.049	<0.001	0.876	0.078	<0.001
		Host on usurped nest	0.703	0.057	<0.001	1.111	0.091	<0.001

Table 4 Intensity and duration of events post-hoc comparisons, Sidak adjustment for multiple comparisons. Mean Diff. = mean difference, SE = standard error, sig = p-value.

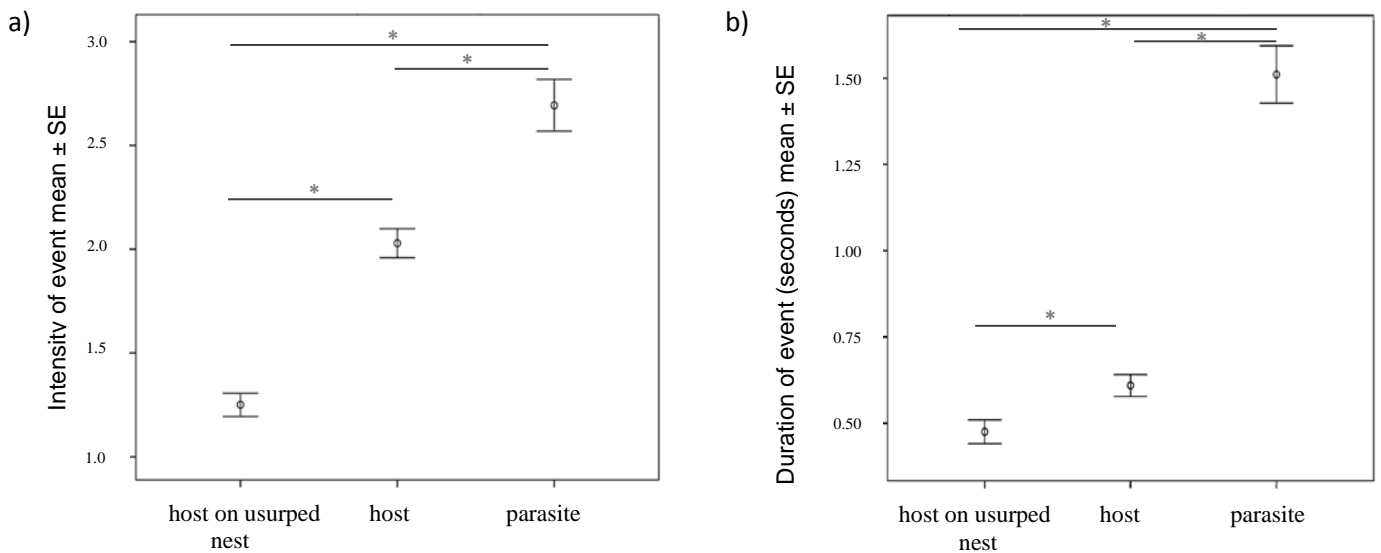


Figure 5 a) Intensity (dimensionless sample units) of vibrational events in host on usurped nest, host in non-usurped nest, and parasite. b) Duration (in seconds) of vibrational events in in host on usurped nest, host in non-usurped nest, and parasite. SE = standard deviation, * = significant difference ($p < 0.05$)

Discussion

This study provided a comprehensive description of the vibrations produced by two oscillation movement, AbW and AbD respectively produced by the host species, *P. dominula* and its social parasite, *P. sulcifer*. Moreover, we demonstrated that even if AbD events had similar structure to AbW (i.e. each event composed of a sequence of broad-band pulses), they were well differentiated according to the intensity of events and their duration.

The previous description of vibrations produced by AbW reported frequencies centered around 904 Hz (Brennan 2007). In contrast, our results showed that most of the energy is concentrated at much lower values of about 50 Hz. This difference is probably due to the sensitivity of the instrument used for recording and by the characteristics of the instrument itself. Contrary to the accelerometer used by Brennan (2007), the laser vibrometer enables to completely avoid any load on the surface on which vibrations are transmitted, therefore to minimize interferences with surface-borne vibrations involved in insect communication (Michelsen & Larsen 1978, Michelsen et al. 1982, Hill & Wessel 2016). Since AbW produce vibrations with a broad-band spectrum, it is possible that only the higher components were detected by previous studies. Moreover, in insects, sensory organs able to detect substrate vibration below 100 Hz are widely spread (Virant-Doberlet & Čokl, 2004). In both adults and immature insects, low frequencies have been demonstrated to be able to determine physiology changes, for instance in the content of juvenile hormone in honeybee workers (Schneider et al. 2004), larvae growth in *Tribolium castaneum* (Hirashima et al. 1993), and larval development in *P. fuscatus* (Jandt et al 2017, Suryanarayanan et al. 2011). Therefore, even if *Polistes* oscillatory movements produce broad-band pulses, it is more likely that low frequency components can play a role on paper wasps nest.

Given the production modality (oscillation of the abdomen on the nest surface) and the wide broad-band spectrum, both AbW and AbD should be considered percussive signals (Elias & Mason 2010). This kind of signal usually provides informations by both temporal (duration and rate of pulses) and spectral (frequencies and intensity) parameters (Hill 2009). One of the characteristic of percussive signals is that the frequency profile is the result of the substrata properties (Pierce 1989). Therefore, it is possible that the paper nest structure itself emphasizes low frequencies. Future studies on the paper material and structure of the nest would be required to understand how much they affect the vibrations produced by oscillatory movements towards similar spectrum features. In this regard, the result of the discriminant analysis seems to support the hypothesis of homogeneity of spectral components proposed by Pierce (Pierce 1989, Elias & Mason 2010). In fact, the variability of frequency features among different contexts and the two

species is not sufficient to distinguish them. On the contrary the duration of events and their intensity accounted for the major differences.

Since AbD can be an exaggeration of the host's AbW, parasite could exploit potential vibrational host signal to communicate to both larvae and adult that on the nest there is a dominant female and are required subordinate and workers females (Jeanne 2009).

A well-studied system in which parasites are known to exploit both chemical and the acoustic host communication is represented by the butterflies of the genus *Maculinea* (Akino et al. 1999, Elmes et al. 2002, Schönrogge et al. 2004, Nash et al. 2008, Thomas et al. 2010, Barbero et al. 2009). These butterflies are obligate social parasites of *Myrmica* ants and use acoustic signals to break their host communication system. The trick of *Maculinea* cuckoo larvae is highly refined as they are able to reach a higher social status within the colony's hierarchy by mimicking the acoustic signals of their host queen ants (Barbero et al. 2009, Casacci et al. 2013). Under this point of view *P. sulcifer*, too, could exploit the chemical and vibrational cues of host dominant females to hold dominant rank position. When *P. sulcifer* usurps host colony, alpha host female dies or leaves the nest (Turillazzi et al. 1990) and, with the loss of dominant foundress, it's lost her control on colony order and her inhibition on subordinate and workers reproduction (Pardi 1946). So, the parasite female replaces dominant foundress by performing exaggerate dominance behaviors toward host females and acquiring alpha chemical profile (Turillazzi et al. 1990, Sledge et al. 20010, Dapporto et al. 2004, Cini et al. 2011b, Cini et al. 2014). In additions of this, the exaggeration of host vibrational signal, could be used by the parasite to bolster chemical exploitation and to impose itself on host adult females present on the nest. We can, also, speculate that the vertical oscillation of abdomen, which characterize AbD, allows parasite to produce more intense vibrations than those produced by horizontal oscillations (AbW); however, further studies on physic and mechanical aspects of vibrations production in wasps are needed to confirm this hypothesis.

Finally, a link between dominant status and vibrational behaviors, suggested by Röseler and Röseler (1989), is partially supported by the comparison between host in usurped and non-usurped nests. Indeed, similarly to what found by Mignini & Lorenzi 2015 in *P. biglumis* in which foundresses on usurped nests perform AbW at lower rate than foundresses on non-usurped nests, *P. dominula* foundresses perform shorter and less intense AbW in usurped nests (in which they are subordinate) than that on non-usurped nests.

However, the hypothesis of host dominance vibration code exploitation does not exclude that vibrations could play a role in adult-brood communication. Supernormal manipulation has been reported and demonstrated for several host-social parasite systems. Usually the immature

brood of the parasite exaggerate begging signals to attract more care from the host adults, such as *Atemeles pubicollis* in nests of the ant *Formica polyctena* (Hölldobler & Wilson 1990) or cuckoo chicks in host nests (Redondo 1993). We suggest an inversion of the supernormal manipulation as it has been demonstrated for other animals: if vibrations modulate the nourishment effect in *P. dominula* caste determination, it is possible that AbD itself is an exaggeration of the host behavior (i.e. AbW) made by the adult parasite to manipulate the host's female brood. In fact, the parasite relies exclusively on the rearing abilities of the host workers that will emerge after the nest usurpation to rear its own brood (Cervo 2006). Therefore, the possibility to manipulate their future behavior as adult workers would be surely beneficial. Our observation, however, not allow us to verify if the parasite performs AbD when in the nest is present also her immature brood and if the latter experiences and is affected by vibrations. Indeed, one possibility is that the parasite female larvae don't experience the AbD because adult parasites perform vibrational behaviors only in early phase when in the nest are present the host larvae and parasite male larvae (Cervo 1990); the second possibilities is that parasites produce AbD vibrations during the entire nest cycle, but the parasite larvae are immune or little affected by vibrations (for example because their biochemical pathways are insensitive to vibrations). Further studies are needed to clarify the effects of vibrations on parasite larvae.

Why the exaggeration is not greater to entail a higher discrimination between AbW and AbD? One possibility is that there is a physical constraint to perform events with higher number of pulses, for example due to the energetic cost of moving the abdomen. A second possibility, which does not exclude the first one, is that the parasite must not stretch too much the features of the host behavior to avoid being recognized as intruder and reject from the colony. In fact, a model developed on the game theory shows that exaggerated signals in social parasite are evolutionary supported only at low rate of parasitism or if they remain below a definite threshold to disable the host to recognize the intruder and thus reject it (Holen et al. 2001). However, the final proof will be to assess the role of these vibrations in *P. dominula* in future experiments, for instance testing different playbacks effect on larval development. Our results suggest that, at constant frequency components, higher number of pulses and rate should be more likely to drive larvae development towards workers phenotype, such as lower fat bodies quantity.

In conclusion, we demonstrated that the parasite species *P. sulcifer* as other *Polistes* species produce surface-borne vibrations and the characteristics of the parasite vibrations suggest that it is a case of exaggerated behavior performed by a social parasite that could "increase" the benefit from usurpation.

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Authors contribution

RC, AC, RN, IP planned the experiment, RN, IP performed the experiment and analyzed spectral data, AC, RN, IP analyzed data, RC, AC, RN, IP wrote the paper, RC provided funds, VM provided instrumental support, all authors read, commented and finally agreed on the MS.

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5. Dominance hierarchy and vibrational communication in social wasps: top rank individuals produce more intense vibrations on the comb in the paper wasp *Polistes dominula*

Irene Pepiciello^{1*§}, Alessandro Cini^{1#*}, Rachele Nieri^{1,2, x}, Valerio Mazzoni², Rita Cervo¹

§ corresponding author: Irene Pepiciello, mail: irene.pepiciello@unifi.it; * equally contributing authors

1 Dipartimento di Biologia, Università di Firenze, Via Madonna del Piano 6, 50019 Sesto Fiorentino (FI), Italy.

2 Dept. of Sustainable Ecosystems & Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, via Mach 1, I-38010 San Michele all'Adige (TN), Italy.

#Current address: Centre for Biodiversity and Environment Research, University College London, Gower Street, London WC1E 6BT

x Current address: Center Agriculture Food Environment, University of Trento, via Mach 1, 38010, San Michele all'Adige (TN), Italy

Abstract

Small societies of primitively eusocial paper wasps of *Polistes* genus lack of morphological differentiation between breeder and helpers and reproductive skew is behaviorally regulated by dominance hierarchies. In multi-foundresses colonies of *P. dominula*, top rank position is initially established by aggressive interactions between foundresses and then is maintained by a series of ritualized dominance behaviors. However, it had been recently reported that chemical and visual cues can take part in establishing dominance hierarchies too. In this framework, here, we investigate the possible involvement of vibrational communication in dominance signaling of this species. In *P. dominula*, a particular behavior, called abdominal wagging (AbW), was described as typically performed by dominant females. It consists of a series of oscillatory lateral movements of the entire abdomen that produces substrate-borne vibrations. In this study, we recorded and analyzed vibrations produced by co-foundresses of different rank and measured morphological and physiological features of such individuals (body and abdomen size, ovary development) to investigate whether temporal and spectral features of AbW are associated with dominance status. Our results show that in *Polistes dominula* the intensity of vibrations produced by AbW is related to dominance status. We found that among all measured morphological and physiological correlates of dominance status, higher intensity of AbW is associated with increased fertility, but not with body size nor relative abdomen size. Our results by adding new insights to dominance behavior in *Polistes* wasps suggest that simple ritualized dominance behavior is probably part of a more complex communicative system where different sensory signals are involved.

Keywords: dominance signal, fertility signal, vibrations, abdominal wagging, biotremology

Introduction

Reproductive skew, i.e. the extent to which breeding is monopolized by dominant individuals, represents the most salient feature of insect societies. How such reproductive partitioning is maintained among members of social groups is a central question in behavioral ecology and indeed regulation of reproductive skew largely varies in different social insects species.

In many highly eusocial insects with very large colonies, such as in many ants, bees and termites, the reproductive role is concentrated in one or a few individuals i.e. morphologically specialized queens that communicate by pheromones their presence and status to workers that cannot mate and are limited in their direct reproduction by queen presence (Winston 1987, Bourke 1988, Keller & Nonacs 1993, Peeters 1993, Johnstone 2000; Van Oystaeyen et al, 2014).

A different way to maintain reproductive skew is found in many insects species with a primitively eusocial structure i.e. small societies lacking morphologically specialized breeders and helpers where reproduction is often restricted to behaviorally dominant individuals (Pardi 1946, Breed and Gamboa 1977, Ross & Matthews 1991, Reeve 1991, Röseler 1991, Hunt 2007). In such societies all adult females have almost equal potential to mate and lay fertilized eggs but usually only the dominant individual does. Reaching and maintaining the top rank position in the hierarchy is thus fundamental for assuring a reproductive success (Breed & Gamboa 1977, Röseler 1991, Ross & Matthews 1991, Monnin & Peeters 1999). In such primitively eusocial insects, dominant behavior and, more generally, the capacity of signaling the dominant rank during interactions between colony members is a crucial feature to regulate these societies. Rank position in a dominance hierarchy is initially determined by overt aggressive interactions, which are usually soon replaced by predetermined conventions based on specific ritualized behavioral patterns, that allow to communicate dominance in a safer communicative way without engaging in risky overt fights (Pardi 1942,1948, Röseler 1991, Jandt et al. 2014). While, traditionally, dominance hierarchy signaling has been reported as a sequence of peaceful tactile behavioral patterns performed by dominant female in a ritualized way on the body of the subordinate, the benefit of rapidly and safely communicating dominance status suggest that it is likely that other sensory channels (i.e. chemical, visual and vibrational ones) could be used to improve and integrate the establishment and the signaling of dominance status (Tibbetts and Dale 2004; Tannure-Nascimento et al. 2008, Leonhardt et al. 2016).

The small and flexible societies of the social paper wasp *P. dominula* represent good models for such study as *a*) linear dominance hierarchy is well known to occur in this species and a large body of work has elucidated many physiological correlates of dominance (Pardi 1948, Jandt et al.

2014); *b*) the species is highly flexible in the use of multiple sensory channels for intra and intercolonial communication (Hunt & Richard 2013, Cervo et al. 2015, see chapter 3).

Since the 1940s, Pardi highlighted the presence of a linear dominance hierarchy in *P. dominula* (Pardi 1948); he described, for the first time in an invertebrate, that in multi-foundress colonies (colonies started by two or more females), after a few days of frequent overt dominant-subordinate aggressive interactions, a stable hierarchy is established. The hierarchy is then maintained thanks to repeated ritualized dominance behaviors performed by the dominant female, who strikes her antennae on the body of subordinate co-foundresses, who assume a submissive position, often performing a trophallactic exchange toward the dominant female (Pardi 1942, 1948).

The establishment of the dominance hierarchy is facilitated by morphological and physiological factors, and also triggers a series of physiological modifications, so that dominant females, which are often larger than subordinates, show better developed ovaries (Pardi 1946, 1948, Gervet 1962; Turillazzi and Pardi 1977), have enlarged corpora allata (CA) and this enlargement is correlated with an increased synthesis of juvenile hormone (JH) (Pardi 1980, Röseler et al. 1980, Röseler et al. 1985, Turillazzi et al. 1982, Röseler 1991, Tibbetts and Izzo 2009). Reaching the status of dominant and breeding female in the nests also triggers modifications in the profile of cuticular hydrocarbons, that are the main recognition pheromones in social insects. Indeed, Sledge et al. (2001) found that in multi-foundresses colonies, dominant breeding females can be distinguished from subordinates on the basis of the proportions of some hydrocarbons on their cuticle. Moreover, Dapporto and co-workers (2007) reported that eggs laid by dominant and subordinate females in *P. dominula* are characterized by a distinctive chemical signature that resemble chemical profile of the correspondent laying individual suggesting the existence of a chemical marker for egg recognition in this species. The cuticular hydrocarbons profile thus assumes in this species the role of a dominance status signaling system, which inform about the dominance and reproductive status of a colony member. In turn, this reinforces the maintenance of the dominance hierarchy and of the reproductive skew, by both physical interactions and egg policing (Dapporto et al 2010).

In addition to behavioral and chemical signaling systems, *P. dominula* foundresses have been suggested to use also visual cues as quality signals (Tibbetts and Dale 2004, reviewed in Cervo et al. 2015). The number, size and shape of black spots on the yellow clypeus of the wasp face is a very variable trait, which, in at least in an invasive North American population of *P. dominula*, has been shown to be positively correlated with body size and to predict social rank (Tibbetts and Dale 2004, but see also Cervo et al. 2015). However, the association between

dominance status and clypeal patterning only holds for a North American population while it has been disproved in European populations (Cervo et al. 2008, Green et al. 2012, Branconi et al. 2017).

The finding that tactile, chemical and visual signaling could be involved in the assessment of status position in the linear hierarchy among co-foundresses suggests that the simple ritualized hierarchy described in *P. dominula* since 1940s is probably part of a more complex system where the exchange of information through multiple sensory channels is involved (Pardi 1942, 1948, Sledge et al. 2001, Tibbetts and Dale 2004)

Another sensory channel which may be potentially involved in dominance signaling is vibrational one. In *Polistes dominula*, a particular behavior was described as typically performed by dominant female; this behavior is an oscillatory lateral movement of the entire abdomen, called abdominal wagging (AbW) (Pardi 1942, Röseler & Röseler 1989), and consists in a wasp that, while standing on the nest, shakes the abdomen side to side and produces substrate-borne vibrations (Brillet et al. 1999, Brennan 2007). Vibrations produced by AbW are characterized by a series of broad-band pulses, corresponding to the moment in which the abdomen beats against the nest (Brennan 2007), with the energy produced by AbW is concentrated on low frequencies (see chapter 4).

Brillet et al. (1999) found that the AbW is performed in numerous contexts, namely during social interactions, nest inspection and feeding larvae. AbW is performed in both single- and multi-foundress colonies and in the latter case AbW is more frequently performed by dominant females than subordinates and it is often temporally associated with aggressive encounters (Brillet et al. 1999). AbW can be considered a percussive behavior (Brennan 2007, Hill 2009, Cocroft et al. 2014). This type of behavior, strictly related to the energetic costs (Jeanne 2009, Elias & Mason 2010), produces a broadband signal that, in temporal (duration) and/or spectral (i.e. intensity) parameters, can contain information about the individual who performs them (Hill 2009). This evidence led to the hypothesis that AbW could be involved in the dominance signaling system and, acting on a series of biochemical pathways, it might control the reproductive physiology of subordinates and larvae (Brillet et al. 1999, Brennan 2007, Jeanne 2009). Some studies have demonstrated that low frequency vibrations are able to determine physiological changes in both adults and larvae in various insect species i.e. *Apis mellifera* (Schneider et al. 2004), *Tribolium castaneum* and *T. freemani* (Hirashima et al. 1993, 1995), *Polistes fuscatus* (Suryanarayanan et al 2011). Interesting, the low frequency vibrations produced by female of *Polistes fuscatus* by drumming her antennae on the rim of the larvae cells are proved (through playback experiments) to be involved in modulating the amounts of fat in the emerging adults, a

trait linked to worker-queen phenotype that affects survival diapause capacity (Suryanarayanan et al. 2011, Jandt et al. 2017). Under this perspective, AbW could be regarded as a component of ritualized dominance behaviors (Röseler & Röseler 1989) with effects on both adults and immature individuals.

Until now, no direct comparisons have ever been made on the temporal and spectral features of vibrations produced by AbW performed by individuals with different position in the dominance hierarchy. To fill this gap, here we investigated whether temporal and spectral features of AbW are associated with dominance status.

For this aim, we used a laser Doppler vibrometer to record the vibrations produced by co-foundresses during AbW behavior performance on multi-foundress nests and we compared temporal (duration of AbW) and spectral features (dominant frequency and intensity) between dominant breeders and subordinate helpers. Moreover, we investigated which, if any, morphological and/or physiological factors, among those associated with dominance status (such as resource holding potential, fertility and weight) influence the characteristics of the vibrations produced by AbW.

Materials and methods

Studied species

Polistes dominula shows a typical colonial cycle of a temperate *Polistes* species (Pardi 1996; Reeve, 1991). Colonies are founded, at the beginning of spring, by one or more mated females that have overwintered in sheltered places. In multi-foundress colonies, after the dominance hierarchy is established, the dominant female avoids performing risky and energetically costly behaviors and lays most of the eggs (Queller et al. 2000) while other females become subordinates and perform all the colonial and extra-colonial work i.e. foraging, nest-building and brood care. This means that the dominant foundress is the female with the highest degree of ovary development whereas the subordinate females show a regression of their ovaries and they do not reproduce but behave as workers (Pardi, 1996). At the end of May, the first workers emerge, starting the post-emergence phase. Workers assume riskier foraging and defensive tasks and help until the end of the season in rearing additional brood. Reproductive individuals, males and gynes, emerge only later in the season, from the end of July. They leave the natal colonies and mate far from their nests (Beani 1996).

Animals collection and laboratory rearing

Multi-foundresses colonies (n = 11) of *Polistes dominula* in pre-emergence phase were collected in the surroundings of Siena and Florence (Italy) at the beginning of May. All colonies

were immediately transferred to the laboratory, where each nest was fixed on the ceiling of a plastic cage (15x25x15 cm) and provided daily with water, sugar and fly maggots *ad libitum*. Prior to mounting the nest in the cage, a reflective sticker was attached on its back surface and used to focus and maximize the sensitivity of the laser beam (see below). All colonies were kept under natural temperature and light conditions with additional illumination from neon lighting. At the moment of recording trials (see below) colonies had on average 48.17 ± 16.62 (mean \pm SD) cells and 3.09 ± 0.70 foundresses (number of foundresses on nests was variable from a minimum of 2 to maximum of 4 females) and inside the colonies were present 18.64 ± 6.62 (mean \pm SD) larvae, 7.27 ± 6.92 pupae and 15.36 ± 9.66 eggs. All wasps of each colony were individually marked with Humbrol enamels on the thorax and/or wings for individual identification. Before recording trials, behavioral observations were carried out for approximately two hours independently by two observers in order to establish the dominant individual for each colony on the basis of the following behaviors: ritualized dominance behavior, egg laying activity, time spent outside the colony and foraging activity (Pardi 1996). Thus for each colony, individuals were classified into two categories: one dominant and reproductive female and one or more subordinate helpers.

Recording trials

Each colony was recorded for a due time to acquire at least one AW event for the dominant individual and one AW event for a subordinate individual (range 8 – 24 minutes). Recording trails were carried out during the central hours of the day (10:00 – 16:00), when wasps are more active on the colony. Individuals' behaviors and vibrations on the combs were recorded simultaneously by means of a video camera (HDD DCR-SR36, Sony Corporation Minato, Tokyo) and a laser Doppler vibrometer (PDV 100, Polytec, Germany) pointed on reflecting sticker previously fixed on nest respectively. The substrate-borne vibrations were digitized with 48 kHz sample rate, 16-bit depth, and stored directly onto a hard drive through a multichannel LAN-XI data acquisition device (Brüel and Kjær Sound & Vibration A/S, Nærum, Denmark). All trials were carried out in a single day at the beginning of May. Immediately after the recording trials all wasps were killed by freezing and then stored at -20°C until they were measured and dissected for recording both morphological and physiological parameters as reported below.

Terminology and vibrations analysis

We define an 'event' as the substrate-borne vibrations recorded with the laser vibrometer while the wasp was oscillating the abdomen on the nest surface (Brennan 2007).

All individuals that performed at least one abdominal wagging (AbW) were considered for the analyses ($n = 11$ dominant foundresses that perform on average 15.09 ± 9.24 AbW (mean \pm SD, with a minimum of 3 AbW and a maximum of 36 AbW) and $n = 17$ subordinates that perform

on average 6.77 ± 6.08 AbW (mean \pm SD, with a minimum of 1 AbW and a maximum of 25 AbW)). Temporal and spectral parameters of AbW events ($n = 166$ and 115 AbW performed by dominant and subordinate foundresses respectively) were analyzed with Raven Pro 1.4 (The Cornell Lab of Ornithology, Ithaca, NY) using Fast Fourier Transform (FFT) type Blackman with window length of 256 samples and 50% overlap. To characterize the AbW we measured duration (i.e. the timespan between the beginning of first pulse and the end of last pulse), dominant frequency (i.e. the frequency with the highest value of energy) and intensity of each event. Since in the previous work (see chapter 4) we found that the number of pulses present in each event and the rate of pulses are correlated with duration of vibrational event here we have considered only duration of events. The intensity of substrate-borne vibrations depends on the distance of the individual producing them from the detection site of recording (i.e. laser beam focused on the nest), which varies within and between different colonies. For this reason, to compare the intensity of AbW, the ratio between the relative amplitude (RMS) (Chariff et al. 2010) of each event and the vibrations produced by walking at the same distance from the laser beam of relative event was measured for each individual. Successively we calculated the mean of each vibrational parameter for each individual to test the influence of social and morpho-physiological parameters on these (see below).

Selection of morpho-physiological parameters

We selected three parameters that are related to dominance status and/or might influence the properties of the produced vibrations (Hill 2008, 2009, Elias and Mason 2010).

Head width (HW): HW has been extensively used as a proxy of overall body size in social wasps and *P. dominula* (Eickwort 1969, Turillazzi & Pardi 1977, Cervo et al. 2008, Ortolani & Cervo 2010) and it has been shown to also be a proxy of individual resource holding potential, i.e. fighting ability in social contexts, in this species (Dapporto et al. 2006, Ortolani & Cervo 2010, Cini et al. 2011, Green & Field 2011). The maximum width of the head was measured under a stereomicroscope at 25x magnification. The whole body and abdomen of each wasp was weighted by means of a GM series professional digital jewelry scale (ALPHA scale Hubei, China). In our sample HW was highly and positively correlated with fresh body weight (Pearson $r = 0.746$, $p < 0.001$, $n = 28$), confirming its validity as body size proxy.

Relative Abdomen weight: To understand whether the weight of the abdomen has an influence on the temporal or structural properties of the produced substrate-borne vibrations, as suggest by Hill (2009) we measured the weight of the fresh abdomen and compiled a relative abdomen weight index as: $\text{RelAbd} = \text{abdomen weight} / \text{total body weight}$. As abdomen weight is positively correlated to both total fresh weight and head size (respectively Pearson $r = 0.889$,

$p < 0.001$, $n = 28$ and Pearson $r = 0.696$, $p < 0.001$, $n = 28$), the RelAbd index allowed us to assess the influence of the abdomen weight on the temporal and structural properties of substrate-borne vibrations independently from the overall body size of the individual (a parameter whose influence is already caught in the HW measure, see above). Relative weight of abdomen is indeed not correlated to head size (Pearson $r = 0.307$, $p = 0.113$, $n = 28$) thus allowing us to consider them as independent measures in the following analyses.

Ovarian development: In order to evaluate fertility status, i.e. investment in reproduction (Cini et al. 2013), we compiled the ovarian development index (OI) as follows: all foundresses were dissected under a stereomicroscope at 25x magnification to measure the length of the six largest/mature oocytes in order to calculate the ovarian index (i.e. the mean length of the six largest oocytes) (Cini et al. 2013). Observation of presence of yolk in the eggs and yellow bodies (Cini et al. 2013; Cini 2014) confirmed the reproductive status of those individuals recognized as dominant (egg-laying individuals, i.e. with yolky oocytes, yellow bodies and at least one egg of layable size, i.e. greater than 1.6 mm, Cant & English, 2006; Cini 2014), and subordinates (poor yolk in oocytes and no yellow bodies) from their behavior.

Dominant breeders are predicted to have higher values for these parameters compared to subordinate helpers, i.e. to be bigger, with more developed ovaries and relatively heavier abdomens (Pardi 1946, Turillazzi and Pardi 1977, Cervo et al. 2008). Our results confirm this prediction (see results section), implicating that it is difficult to disentangle dominance status from morpho-physiological parameters. We then performed two analyses:

1) Firstly, we first perform three separate linear mixed models (Type III sums of squares) to assess whether dominance rank (i.e. being a dominant breeder vs a subordinate helper, considered as fixed factor) significantly affected each of the three parameters of AbW, i.e. the temporal (duration) and spectral (dominant frequency and intensity) parameters of the vibrations (defined below). Nest was included in the model as a random factor.

2) Then, we run the same analysis considering as explanatory variables the three morphological and physiological measures: HW, RelAbd and OI (instead of the dominance category as above). Nest was included in the model as a random factor.

As AbW duration and dominant frequency distributions were not different from a normal distribution (respectively Shapiro Wilk test, $W = 0.958$, $df = 28$, $p = 0.309$ and $W = 0.136$ $df = 28$, $p = 0.944$) we directly used these variables as target variables in the models, while AbW intensity was log-transformed to meet normality assumption (Shapiro Wilk test, $W = 0.274$, $df = 28$, $p = 0.956$). Comparisons of HW, RelAbd and OI between dominant and subordinate individuals have

been performed through linear mixed model, with category as fixed factors and nest as random factor.

As previous reported for other Italian populations of this species, in our sample we did not find any significant association between facial pattern (coded, as in Tibbetts & Lindsey 2008 in three categories, i.e. no spot, one black spot and two or more spots) and dominance status, i.e. dominant breeder or subordinate helper (chi-square $\chi^2 = 0.236$, $df = 2$, $p = 0.889$). This result confirms that clypeal patterning is not associated with dominance status in the studied populations and we thus excluded it from our analyses.

Results

Dominant breeders and subordinate helpers differed for the three morpho-physiological parameters. As expected, dominant breeders were bigger (HW: (mean \pm SE) dominant females = 0.797 ± 0.015 , subordinates = 0.741 ± 0.012 ; $F = 8.443$, $p = 0.009$), with relatively heavier abdomens (RelAbd: (mean \pm SE) dominant females = 0.407 ± 0.016 , subordinates = 0.353 ± 0.013 ; $F = 6.873$, $p = 0.014$) and with more developed ovaries (OI: (mean \pm SE) dominant females = 1.990 ± 0.056 , subordinates = 0.486 ± 0.046 ; $F = 440.667$, $p < 0.001$).

Intensity of vibrations produced by dominant breeders was significantly greater than those of subordinate helpers (relative intensity is (mean \pm SD) 4.71 ± 3.34 and 2.68 ± 1.67 for AbW performed by dominant foundresses and subordinates respectively), while dominance rank had no effect on AbW duration (duration is (mean \pm SD) 0.50 ± 0.37 seconds in dominant females and 0.48 ± 0.30 seconds in subordinates) nor on dominant frequency (the dominant frequencies were (mean \pm SD) 112.39 ± 53.98 Hz and 121.88 ± 52.75 Hz in dominant and subordinate females respectively) (Table 1, Fig.1).

Target variable	Source	Numerator df	Denominator df	F	Sig.
Dom Freq	Intercept	1	9,989	37,488	<0.001
	Dom Rank	1	16,510	0,771	0,392
Duration	Intercept	1	9,548	233,429	<0.001
	Dom Rank	1	17,417	0,737	0,402
Intensity	Intercept	1	10,418	341,675	<0.001
	Dom Rank	1	18,047	6,061	0,024

Table 1 Influence of dominance rank on temporal and spectral parameters of AbW. Results of linear mixed model (Type III sums of squares). Significant explanatory variables in bold. Dom Rank = dominant rank, Dom Freq = dominant frequency, Sig = p-value.

When looking at morphological and physiological parameters, ovarian development was the only factor influencing intensity of vibrations, with wasps with more developed ovaries producing more intense vibrations. Head size and relative weight did not have any effect (Table 2, Fig. 1). Nor the dominant frequency nor the duration of the AbW were significantly influenced by any of the morpho-physiological parameters, even if a trend for an influence of the relative abdomen weight on dominant frequency has been found (Table 2), with relatively heavier abdomen determining lower dominant frequency.

Target variable	Source	Numerator df	Denominator df	F	Sig.
Dom Freq	Intercept	1	16,137	1,536	0,233
	Ovarian Index	1	15,178	0,030	0,865
	Relative Abdomen weight	1	15,442	3,884	0,067
	Head Width	1	15,389	0,512	0,485
Duration	Intercept	1	19,854	0,009	0,924
	Ovarian Index	1	18,307	0,031	0,863
	Relative Abdomen weight	1	19,465	0,290	0,596
	Head Width	1	20,386	0,713	0,408
Intensity	Intercept	1	19,773	5,254	0,033
	Ovarian Index	1	18,604	8,611	0,009
	Relative Abdomen weight	1	19,561	0,328	0,573
	Head Width	1	20,159	0,630	0,437

Table 2 Influence of morphological and physiological features on temporal and spectral parameters of AbW. Results of the linear mixed model (Type III sums of squares) Significant explanatory variables in bold. Dom Freq = dominant frequency, Sig. = p-value.

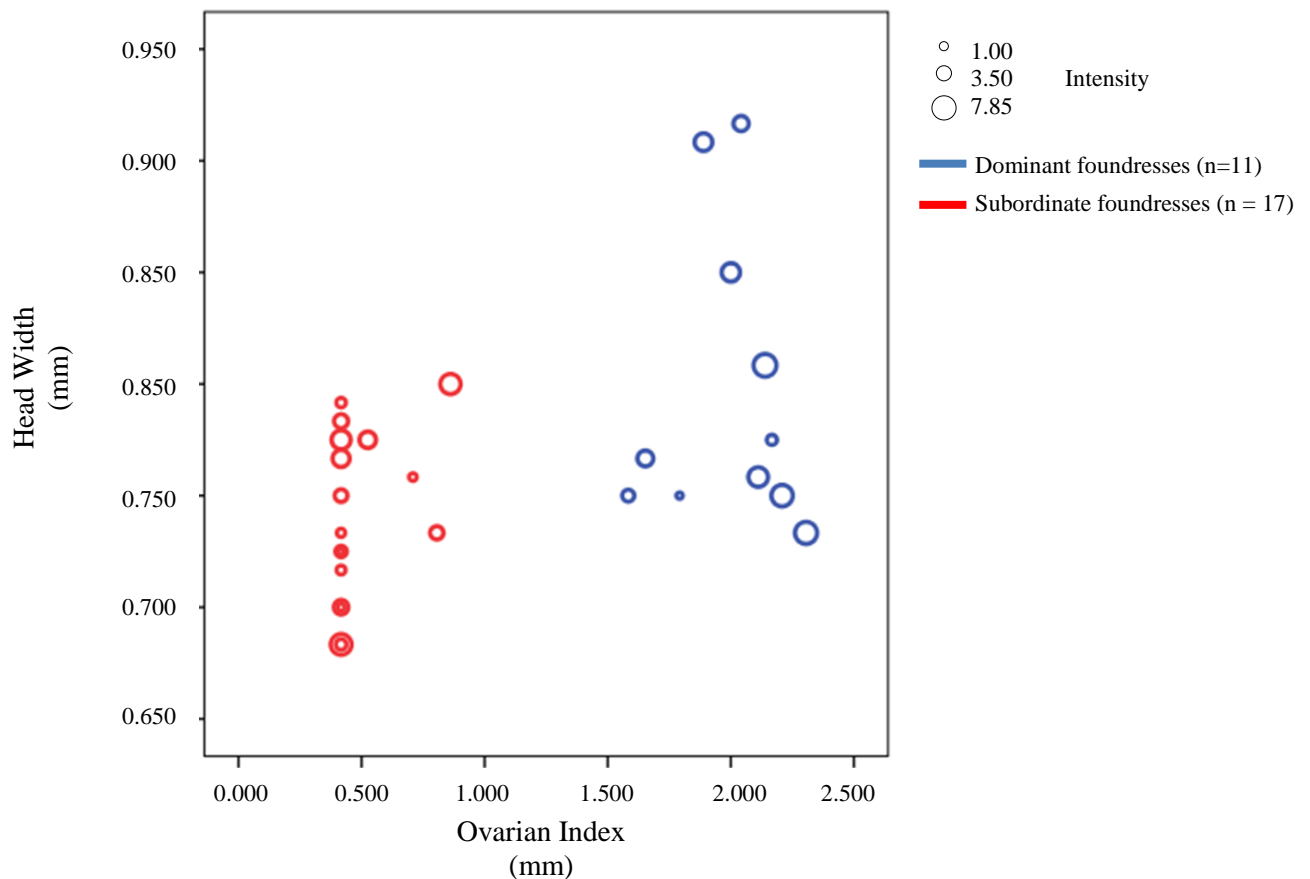


Figure 1 Distribution of recorded individuals along two axes that report ovarian index (millimeters) and head width (millimeters). In graph each individual is represented by a circle. The diameter of circles is proportional to the average of intensity of vibrational events performed by each individual.

Discussion

Our results show that the intensity of vibrations produced by AbW is associated with dominance status in the social wasp *Polistes dominula*. This finding is relevant as it provides the first experimental evidence that, in *P. dominula*, intensity of AbW vibrations could bring information about the individual that produce them. In particular, the intensity of AbW-produced vibrations is higher in dominant breeders compared to subordinate helpers. Moreover, among the morphological and physiological correlates of dominance status, such as body size, fertility and abdomen size, higher intensity is associated with increased fertility, but not with resource holding potential nor relative abdomen size. This result for the first time underlines that, at least one of the spectral features of abdominal wagging, i.e. intensity of substrate-borne vibrations, vary among different foundresses and that this variation is related to a crucial characteristic that is well known to be linked to rank position that each individual held in the hierarchy (Pardi 1946, 1948, Gervet 1962; Turillazzi and Pardi 1977, Revec 1991, Cervo et al. 2008).

This association between fertility and AbW behavior intensity is intriguing, as it might suggest that AbW is an honest fertility signal. However, fertility and dominance are strongly correlated in queenright un-manipulated colonies of *P. dominula*, so that disentangling the relative role of each parameter is difficult and future studies should experimentally decouple fertility from dominance, as already done for chemical signaling (Dapporto et al. 2007).

Honest signals are predicted to be evolutionary stable if they are intrinsically linked to the property they are providing information about or if they are costly to produce, both for production or maintenance costs, thus assuring that individuals of low quality are unable to produce the same signal as individual of high quality (Zahavi 1975). The hypothesis that ovarian development intrinsically (physically) influences the intensity of the vibrations is rather unlikely, given that the relative weight of the abdomen does not influence intensity. However, we can speculate that, as demonstrated in wolf spiders (Hill 2008, Elias & Mason 2010, Kotiaho et al. 1998), also in *Polistes* the energy demand in producing percussive signals is high; which might suggest that, despite the fact that all foundresses can make all intensity range of AbW, only high quality individuals might be able to bear the costs of repeatedly performing an intense signal. Future manipulative experiments will be crucial in understanding the costs of performing such percussive signals in *Polistes* wasps.

Among all the analyzed features of the vibrations produced by AbW behavior only intensity differs between dominant and subordinate individuals. Although the biological meaning in producing higher intensity vibrations needs future investigations, it is possible that dominant breeder by producing more intense vibrations acts more on the low frequency band pulses where the energy is concentrated by allowing vibrations to reach all the potential receivers present on the nests. As low frequency vibrations potentially can induce physiological changes linked to reproductive potential and caste fate in receivers (Suryanarayanan et al. 2011, Jandt et al. 2017), the increase of vibration intensity could assure the dominant female to maintain the full colony control. On the other hand, we did not find any difference in temporal feature of vibrations between dominant breeders and subordinate individuals. Probably, the energetic cost to produce vibrations for a longer time is too high compared to that need to increasing intensity. Moreover, no difference in dominant frequency between dominant and subordinate individuals has been found; probably because, in percussive signals, such as AbW, the distribution of energy along frequencies range depends mainly by the features of substrate that acts as filter by reducing individual level differences (Pierce 1989, Elias and Mason 2010). Finally, we found that body and abdomen size do not influence any of considered spectral parameters. This could be due to the fact that AbW is a lateral vibration of abdomen and some spectral parameters could not be strictly dependent to

individual/abdomen weight. Another possible explanation is that light abdomens could oscillate more easily and more vigorously than heavy abdomens, the sum of active force (muscular force of wasp) and passive force (weight) could reduce the difference in spectral parameters. Future manipulative investigations will allow us to understand more about the biological meaning of spectral features and the amplification of intensity.

Since AbW behavior linked to social interactions is performed by dominant females during the whole colony cycle, and even more in post-emergence period (West Eberhard 1969, Brillet et al. 1999), the vibrations produced by AbW could help to maintain an already established hierarchy as it has been proposed by Brillet et al. (1999). From this point of view, it has been hypothesized (West Eberhard 1969, Brillet et al. 1999) that AbW could represent an extension of tactile signals and physical and aggressive contacts involved in ritualized dominance behaviors and the increase of intensity of the vibration produced by the dominant female goes in this direction. Similar function of substrate borne vibrations has been hypothesized and partially proved in honey bees where new queens produce “tooting” vibrations to whom the other emerging queens, which are still enclosed in their cells, respond with a “quaking” vibrations (Michelsen et al. 1986, Grooters 1987, Kirchner 1993). These types of vibrations seem to be used by newly emerged queens to estimate the number and strength of the competitors and to reduce aggressive behaviors in workers and queens during social contacts (Grooters 1987, Kirchner 1993, Gilley & Tarpay 2005, Gilley 2001). As suggested by “tooting” vibrations in honey bees, that are used by the first emerged queen to increase her chance of success by delaying the emergence of other queens (Grooters 1987), vibrations produced on *Polistes* nest could not only be involved in adult – adult communications but also in adult – brood communications. By signaling also to larvae her presence on nest, the dominant and laying female prepares them to their future status of subordinates, i.e. workers (Brillet et al. 1999, Jeanne 2009). In this perspective, as suggested by recent studies on *P. fuscatus* and *P. biglumis* (Suryanarayanan et al. 2011, Mignini & Lorenzi 2015, Jandt et al. 2017), vibrations produced by oscillatory behaviors could bias larval development in addition to nutritional status, integrating or modulating the effects of the latter.

Overall our findings, for the first time, strongly support a role of the vibrations produced by AbW as a dominance status signal as one of their spectral parameters, intensity, is associated with dominance status and correlated to fertility, suggesting that such features could carry individual information. Our results indicate that high ranked wasps, characterized by well-developed ovaries (Pardi 1948, Reeve 1991, Cervo et al. 2008), could use vibrations, in addition to other sensory channels (Sledge et al., 2001) in social contexts to communicate their status and presence.

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Authors contribution

RC, AC, RN, IP planned the experiment, RC, RN, IP performed the experiment and IP analyzed spectral data, AC, IP analyzed data, RC, AC, IP wrote the paper, RC provided funds, VM provided instrumental support, all authors read, commented and finally agreed on the MS.

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6. Adult-larvae vibrational communication in paper wasps: the signal value of abdominal wagging in *Polistes dominula*

Irene Pepiciello^{1*}, Alessandro Cini^{1,2*,§}, Rachele Nieri^{1,3,x}, Valerio Mazzoni³, Rita Cervo¹

1 Dipartimento di Biologia, Università di Firenze, Via Madonna del Piano 6, 50019 Sesto Fiorentino (FI), Italy.

2: Centre for Biodiversity and Environment Research, University College London, Gower Street, London WC1E 6BT

3 Dept. of Sustainable Ecosystems & Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, via Mach 1, I-38010 San Michele all'Adige (TN), Italy.

x Current address: Center Agriculture Food Environment, University of Trento, via Mach 1, 38010, San Michele all'Adige (TN), Italy

§ corresponding author: Alessandro Cini, mail: cini.ales@gmail.com; ORCID iD: 0000-0003-0355-2188

* equally contributing authors

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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 paper 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 to 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 adult and larvae by modulating salivary secretion (decreasing its amount, in order to prepare larvae to receive food, or stimulating the release of larval saliva to adults). Here, by using an electro-magnetic mini shaker, we assessed, for the first time, the short-term effect of abdominal wagging on larval behavior by recording larval response and by measuring the amount of saliva released immediately after abdominal wagging playback. Our results show that larvae a) are able to perceive the substrate-borne vibrations produced by abdominal wagging and b) react by increasing the movement of their body, possibly in order to attract the attention of adult females during feeding nest inspection. Yet, vibrations neither increase nor decrease the release of larval saliva. Our results support the alleged role of vibrations in adult–larvae communications; however, do not support the long-lasting hypothesis of salivary release modulation.

Keywords: Substrate-borne vibrations, adult-brood communication, playback experiment, larval saliva, larval behavior

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 relatively large unexplored use of vibrational signals in many animal groups, and especially in complex insect societies (Cocroft & Rodriguez 2005, Cocroft et al. 2014, Hunt & Richard 2013). 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 & Schneider 2000, Hunt & Richard 2013). While many studies have investigated and 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 Richards 2013). Although the use of airborne sound playbacks has a long history in ethological studies of diverse taxa (McGregor 1992), substrate-borne vibrations playbacks have been conducted on a limited number of species of social insects (Kirchner 1993, Hölldobler et al. 1994, Evans et al. 2007).

The play-back of vibrations previously recorded on a substrate allows to switch from a 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 intracolony communication in insect societies (Evans et al. 2007, Hager & Kirchner 2014, Suryanarayanan et al. 2011a, Jandt et al. 2017).

Paper wasps (Hymenoptera: Vespidae; Polistinae) represent a group of social insects where the use of substrate-borne vibrations is largely 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 likely producing substrate-borne vibrations. In many species of genus *Polistes* such as *P. fuscatus*, *P. metricus* and *P. canadensis*, body oscillations can be distinct in three oscillatory

behaviors: ‘antennal drumming’ (AD), ‘lateral vibration’ (LV) and ‘abdominal wagging’ (AbW) (Gamboa & Dew 1981, West-Eberhard 1986, Savoyard et al. 1998). Antennal drumming consists in the wasp beating its antennae on the cell rims and it is performed by females on the nest during food distribution to larvae (feeding context) (Pratte & Jeanne 1984). Lateral vibration and abdominal wagging and AbW are instead generally performed during adult-adult interactions and cell inspection (non-feeding context); LV consists in the wasp standing on the comb shaking the abdomen so vigorously that in some cases the oscillation can involve the entire body and produces an audible sound (Gamboa & Dew 1981, West-Eberhard 1986, Savoyard et al. 1998); AbW consists in a longer and slower lateral vibration of the abdomen performed by the wasp while walking over the cells (Gamboa & Dew 1981). Although two or all three kinds of these behaviors can be present in the same species (Gamboa & Dew 1981, Harding & Gamboa 1998), in one of the most studied species of the genus, *Polistes dominula* (Christ, 1791), only one type of body oscillation is known. It 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*, Jeanne 2009, Brennan 2007). In *P. dominula* AbW is performed not only in the non-feeding context, but, similarly 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 pulses’ energy is concentrated on low frequencies (Brennan 2007).

Since 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 it is usually performed during nest inspection (Brillet et al. 1999, Brennan 2007), a behavior that include adult-larvae contact and food exchange (Brennan 2007), it has been suggested to represent an adult-larvae communication signal (Jeanne 2009). However, the function of AbW represents a long-standing and still ongoing debate in wasp sociobiological studies (Gamboa et Dew 1981, Gamboa et al. 1990, Savoyard et al. 1998, Brillet et al. 1999, Brennan 2007, Ishikawa et al. 2011, Mignini & 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 secretion of saliva from larvae to soliciting adults (*salivary release*

modulation hypothesis). Larval saliva is indeed rich in sugars and amino acids secretion and represents an attractive nourishment source 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 the maintenance of sociality in wasp (Hunt 1991). Since both AbW and salivary exchange from larvae to adult are frequent during cells inspection (Hunt et al. 1982, Hunt 1991, Brillet et al. 1999, Brennan 2007), AbW could represent a request of saliva by adult to larvae, in which case vibrations would stimulate the release of this nourishing secretion (*modulation by increase*) (Savoyard et al. 1998). An opposite hypothesis proposes instead that AbW 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 on 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 with a *modulation by increase*, as adult females are indeed unable to imbibe larval saliva and simultaneously keep food between the mandibles (Pratte & 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).

A second (but not mutually exclusive) hypothesis proposes that AbW might have long term effect on larval phenotype, eventually influencing caste fate. The idea is that vibrations would have an interference with the developmental pathway of larvae, which would be biased toward the worker phenotype (Jeanne 2009, Mignini & Lorenzi 2015). The vibration effect on the phenotype has been indeed recently demonstrated for *P. fuscatus*, in which AD on cell rims produces vibrations that interfere with 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 function of AbW is thus contrasting and mainly correlative. To our knowledge, no direct tests of abdominal 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) if 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); b) if 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 it is known that larval responsiveness to vibrational stimuli might change according to larval developmental stage

(Brillet et al. 1999, Suryanarayanan et al. 2011a, 2011b). Moreover, since 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 colonial cycle to evaluate whether larval response differs between the pre- and post-emergence phases. Our experimental design allowed us *i*) to evaluate the signal function 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

P. dominula 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 & Palagi 2006).

Animal collection and laboratory rearing

P. dominula colonies ($n = 60$) were collected around Florence, 33 of them in pre-emergence phase, at the beginning of May, and 27 in post-emergence phase (before the beginning of the emergence of reproductive individuals) at the beginning of July. All colonies were transferred to the laboratory where they were reared, in glass cages (15x15x15 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 \pm SD) 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 for post-emergence ones. All colonies had brood of various stages (i.e. pupae, larvae and eggs). Colonies allocated to different treatments (AbW, WN, C, see below) did not differ for any of the investigated parameters, nor in early pre-emergence group nor in post-emergence group (Kruskal Wallis test, $p > 0.05$ in all cases, see Table S1 in Supplementary Materials). Larvae used in the experiment were classified into two size classes: medium size (corresponding to 3rd and 4th instar) and large (corresponding to 5th instar). First and second instar larvae were not included in the study as it was not possible neither to collect saliva nor 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. workers 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 two 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 no difference between pre- and post-emergence phase larval behavior and in the amount of saliva released was due to differences in food and liquid provisioning.

Playback trials

Colonies (n = 60) were randomly allocated to one of three groups which 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 (**C**) 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 authors' personal recording inventory (see Suppl. Mat. Table S2). AbW was two-minutes long with a rate of 2.5 events per minute (i.e. AbW events regularly spaced 26 seconds from each other). WN (frequency range 0-5000 Hz) was built starting from AbW, by replacing AbW events with WN events of identical duration and intensity.

Playbacks were transmitted by an electro-magnetic shaker (CBC Europe, Milan, Italy) that was fastened to a plastic wire (\varnothing 0.4 cm) using a U-shaped metal screw which was inserted into the shaker's tip (see Fig. S1 in Supplementary Materials 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 & Vibration A/S, Nærum, Denmark) was used to ensure that the AbW and WN playbacks were correctly transmitted to the nests and resembled the natural occurring AbW produced by a female wasp (table S1, Supplementary materials). Recordings were digitized with 48 kHz sample rate, 16-bit depth, and stored directly onto a hard drive through a multichannel LAN-XI data acquisition

device (Brüel and Kjær Sound & Vibration A/S, Nærum, Denmark). Spectral features were analyzed with Pulse 14.0 (Brüel and Kjær Sound & Vibration A/S, Nærum, Denmark) after applying fast Fourier transform (FFT) with a window length of 400 samples and 66.7% overlap and Blackman–Harris window.

Twenty-four hours before the playback experiment proteic 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

The behavioral response of larvae during the two-minutes of playbacks was recorded through a video camera pointed on the cells opening side of 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 & Gamba, 2016) to annotate larval behavior. For each larva we recorded the number of movements it performed, considering two classes of movements: a) movement of the entire body, i.e. lateral and/or up-down movement of the body, excluding movement of the palpi, b) palpi movement, i.e. opening and/closing of the mouth by moving the palpi. Video recordings were annotated without knowing which treatment they belong to. Only those larvae who 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 number of larvae tested per nest \pm st.dev = $3,8 \pm 1.3$).

To assess that the baseline behavior of larvae (body movements and palpi movements) was not different among colonies allocated to different treatments (AbW, WN and C), we video recorded colonies also one minute prior the treatments and we analyzed behaviors in the same manner.

Saliva collection

To evaluate the effect of vibrations on larval saliva release, the latter was collected immediately after the end of two minutes of playback following the protocol used in Turillazzi et al. (2004): briefly, we gently stimulated mouthparts of each larva with a 5 μ l calibrated transparent glass microcapillary (Blaubrand®) and we recorded the volume of saliva released after one minute of stimulation.

Preliminary analyses were carried out to infer the proper time interval of stimulation to sample saliva. 15 larvae from two nests were stimulated on the mouth with a 5 μ l calibrated transparent glass capillary tube and the amount of saliva released was recorded every 30 seconds for 3 minutes. The amount of saliva released rapidly dropped down as time passed, being the

largest part of the total amount released by larvae within the first 60 seconds (90.4 ± 10.8 % (mean \pm SE) of the total amount, Fig. S2). In the first 30 seconds the amount of released saliva was smaller (on average, 79.0 ± 18.0 % mean \pm SE) but the coefficient of variation was greater (22.8 compared to 11.9 for the 60 seconds interval). So, we performed analysis at both 30 seconds and 60 seconds to increase the likelihood of detecting any effect of the treatment. As the results are largely overlapping, we reported only the results relative to the 60 seconds interval (those related to the 30 second interval can be found in Supplementary Material, S5). Overall, 282 larvae from 59 colonies were tested (mean number of tested larvae per nest \pm st.dev = 4.70 ± 1.47).

Statistical Analyses

Non-parametric one-way ANOVA (Kruskal Wallis test) was used to assess for any difference in terms of number of cells, number of adult wasps and number of brood among colonies allocated to the three treatments (AbW, WN, C), 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, C) before the playback experiment. To assess the influence of treatment, colonial 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 equations (GEE) approach, which extends the generalized linear model to allow for 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, Hubbard et al., 2010, Overall & Tonidandel 2004). 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 the body and b) the number of times a larva was seen moving the palpi. For both GEE, Treatment, colonial 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 number of movements (results about binarized data can be found in Supplementary Material, Table S3 and S4).

To assess the influence of treatment, colony stage and larval size on saliva release we used GEE, using 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, $D = 0.047$, $df = 282$, $p > 0.200$). The amount of saliva released (log-transformed) by each larva was the dependent variable, while treatment (AbW, WN, C), colonial stage (pre-emergence vs post-emergence) and larval size (large or medium-size) 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 were recorded from each colony). To calculate the effect size we computed Cohen's d as: $d = \frac{m_a - m_b}{sd}$ where m_a and m_b are the estimated marginal means of each category within the pairwise comparison, and sd 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 (Table 1-2, Figure 1)

Before the stimulation with the playback, larvae were almost motionless and no difference in likelihood of moving was observed in larvae on nest allocated to different treatments (body movements: average is 9.13%, $\chi^2 = 3.82$, $df = 2$, $p = 0.148$; palpi movements: 5.20 %, $\chi^2 = 0.813$, $df = 2$, $p = 0.666$).

When subjected to vibrational playback experiment, the number of body movements performed by larvae was influenced by vibrational treatment, larval size and colonies phase (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 vs WN: $p = 0.010$, effect size $d = 0.46$) and those subjected to C ($n = 67$) (9.90 times more; $p = 0.007$, effect size $d = 0.95$) (Fig. 1a). Larvae subjected to WN moved 4.87 times more than those subjected to C ($p < 0.001$, effect size $d = 0.67$) (Fig. 1a).

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

Source	Type III		
	Wald Chi-Square	df	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 x Treatment	0.960	2.000	0.619
Treatment x Larval size	1.630	2.000	0.443
Colony phase x Larval size	1.140	1.000	0.286

Table 1 The influence of treatment, colony phase and larval size on numbers of larvae body movements. Results from Generalized estimating equation. Significant explanatory variables in bold.

On the contrary, when considering as a variable the number of times a larva moved the palpi, no significant effect was detected for any factor nor for any interaction of factors, except for a significant effect of larval size (Table 2, Fig.1; large size larvae moved palpi about 2.78 times more than medium-size larvae, $p = 0.022$, effect size $d = 0.36$).

Source	Type III		
	Wald Chi-Square	df	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 x Treatment	0.606	2.000	0.739
Treatment x Larval size	1.160	2.000	0.560
Colony phase x Larval size	0.030	1.000	0.862

Table 2 The influence of treatment, colony phase and larval size on numbers of palpi movements of larvae. Results from Generalized estimating equation. Significant explanatory variables in bold.

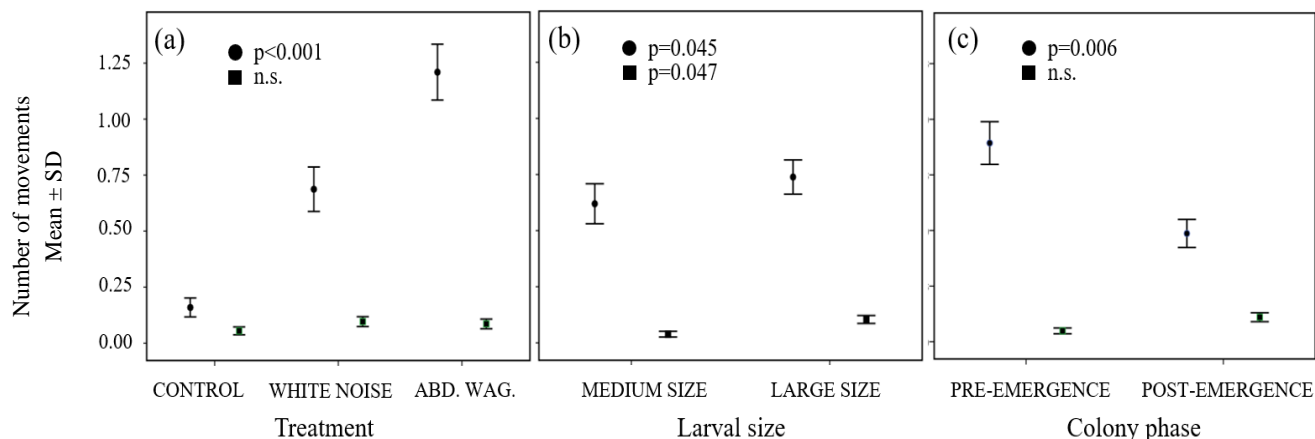


Figure 1 Effect of a) treatment (ABD.WAG.= abdominal wagging), b) larval size and c) colony phase on the body and palpi movements of larvae. Means \pm standard deviation (SD) are reported for the number of body movements (circles) and the number of palpi movements (squares). ns = not significant difference.

Saliva release experiment (Table 3, Figure 2)

Different treatments (AbW, WN, C) had no effect on the amount of saliva released by stimulated larvae, while both colonial stage and larval size had a significant effect (Table 3, Fig. 2): larvae from pre-emergence colonies released on average 1.38 time more saliva than larvae from post-emergence colonies ($p < 0.001$, effect size $d = 0.48$, $n = 123$ vs 159 larvae from pre- and post-emergence colonies respectively, Fig. 2c); large larvae release on average 1.22 time more saliva than medium-size larvae ($p = 0.001$, effect size $d = 0.38$, $n = 182$ large-size larvae vs 100 medium-size larvae Fig. 2b).

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 3 The 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.

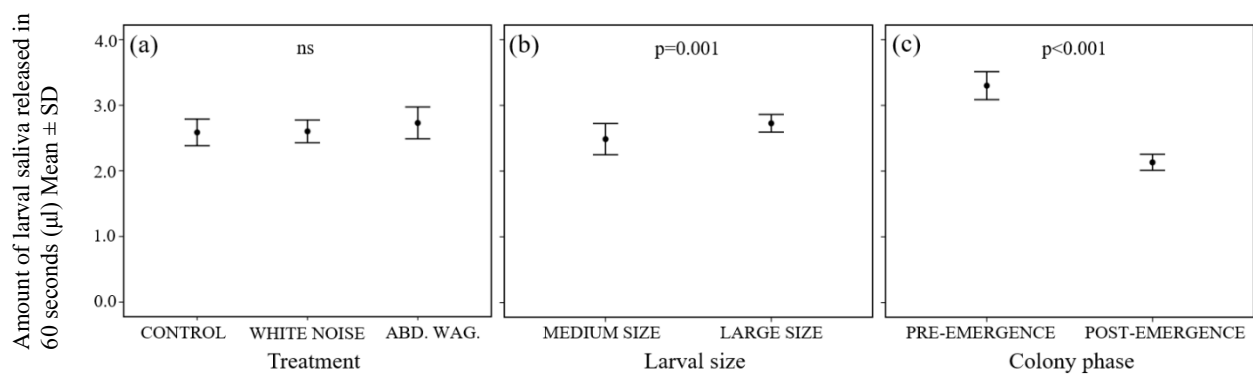


Figure 2 Effect of a) treatment (ABD.WAG.= abdominal wagging), b) larval size and c) colony phase on the amount of saliva released by larvae in 60 seconds. Means \pm standard deviation (SD) are reported. ns = not significant difference.

Discussion

Experiments conducted to stimulate *P. dominula* larvae with substrate-borne vibrations played back onto paper nest 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 demonstrate, 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 putative vibrational signal previously recorded without the physical presence of the signaler allowed us to exclude that larvae were responding to any pheromone released from some abdominal glands in association with abdominal wagging. Chemical communication is predominant in social insect societies and the spread of olfactory signals are often accompanied by a variety of specific behaviors and movements that favor the emission and diffusion of chemicals (Vander Meer et al. 1998, Vander Meer & Alonso 1998). Playback experiments that rebroadcast vibrational natural signals on the colonies and observe the response of colony members give us the opportunity to discriminate among behavioral patterns linked to different communicative channels and so to deepen our knowledge on 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 adult 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 (for hornets: Ishay & Ikan 1968, Ishay & Landau 1972, Ishay & Brown 1975, for ants: Cassill & Tschinkel 1995, Kaptein et al. 2005, for bumble bees: Pereboom et al. 2003, for

honeybees: Huang & Otis 1991, Heimken et al. 2009), but the opposite, i.e. that that adults produce signals to communicate with larvae, has been poorly investigated and our results open a new intriguing research avenue.

An outstanding question is what the function of such adult signals is. Our experimental approach contradicts the *salivary release modulation hypothesis* proposed for this behavior (Savoyard et al. 1998, Brennan 2007, Jeanne 2009). Larvae that experienced the vibrations produced by AbW (track) did not change the amount of released saliva with respect to control larvae, therefore suggesting that AbW vibrations have no effect in regulating larva saliva release, as stated by Brennan (2007) and partially supported by correlative evidence in the American species *P. fuscatus* (Savoyard et al.1998, Cummings et al. 1999). The finding that no difference exists between the no-vibration control and the white noise control (where non-specific vibrations were given) suggests that not only the vibrations produced by AbW had not that function, but that in general non-specific vibrations themselves do not seem to influence salivary release. Salivary release was instead influenced by two factors: larval size and colonial stage. As expected, large larvae released more saliva than medium-size 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, that would change with overall size (Suryanarayanan & Jeanne 2008). Regarding colony stage, larvae from pre-emergence colonies released more saliva than larvae from post-emergence colonies, independently from larval size.

An intriguing potential explanation could be linked to the receivers of saliva. In pre-emergence colonies the only saliva receivers are represented by the queen (larvae’s mother) and subordinate foundresses (who 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. basically non-reproductive individuals. As saliva production is an energetic 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 colonial 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-Eberhahrd 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 hardly 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, has been hypothesized by Hunt (2007) and is strongly supported by our data.

On the other hand, behavioral results of our playback experiments suggest a role related to the feeding context of AbW. General body movement was not the same when larvae were stimulated with AbW track or with controls. First, in general, vibrations provoked a higher degree of body movement (lateral and vertical) by larvae. More interestingly, however, the specific vibrations produced by AbW elicited more movements (roughly the double) than those elicited by the a-specific white noise track. This suggests that the specific vibrations of AbW are perceived and evoke a behavioral response in larvae. This observation suggests the need for further investigations about the possible role of temporal and spectral parameters of AbW in the degree of behavioral response. It is known that even apparently small differences in vibrational signals parameters (i.e. frequency span, intensity) can produce significant differences in terms of behavioral responses of stimulated individuals in insects (e.g. Suryanarayanan et al., 2011a; De Groot et al. 2011; Mazzoni et al. 2015; Polajnar et al. 2014). While we currently have no experimental evidence regarding the actual function of the larval movement, as body movement should be energetically costly for larvae, we can speculate on the possibility that larval movements represents response signals to the adults' call, in order to attract the attention of adult females during the feeding and nest inspection context, to 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). Overall, our results show that larvae perceive vibrations and thus that AbW is indeed a specific vibrational signal produced by adult females and perceived by larvae, which respond to this in a measurable way.

As well as the release of saliva, also larval movement were influenced by larval size and colony stage. Large larvae moved more than medium-size ones. This is explainable by considering the more advanced developmental stage of large larvae, which might need a higher degree of cares (e.g. food) by adults and/or might be more able to move. Larvae from pre-emergence colonies were moving 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 only or the few adults present on the colony. After workers emergence, this adult-attraction need 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. Probably, to reduce energy costs, each larva moves the palpi only at the moment in which a female after returning on nest with food, insert the head into the larva cell.

An alternative hypothesis about the function of AbW is related to its possible long-term effect on behavior and physiology of larvae. Even if the physiological mechanisms which 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 & 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, so to maintain hierarchy on the nest and to prepare the larvae to their future status of workers. In this way, vibrations could trigger physiological events in larvae driving caste development toward a worker phenotype, as it has been demonstrated in *P. fuscatus* with antennal drumming (Suryanarayanan et al. 2011a, Jandt et al. 2017). We did not test any long-term effect, but this hypothesis might explain why AbW is more common in dominant than subordinate individuals and why is more frequent in the early than in late phase of colony cycle (Savoyard 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 the AbW are perceived by larvae and elicit in them a behavioral response, thus qualifying AbW as a signal and supporting a role for AbW in adult-larval communication, a role largely corroborated by correlative, but so far no direct, evidence (Brillet et al. 1999, Brennan 2007, Mignini & Lorenzi 2015); and *ii*) dismiss 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, nor as an increase or a decrease of it, as previous authors suggested (Brennan 2007).

Future studies will use playback to assess 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). Our observations contribute to the growing body of evidence (Hunt & Richard 2013, Cervo et al. 2015) that communication channels others than chemicals have an important role in regulating insect social life.

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Authors contribution

AC, IP, RC, RN, planned the experiment, AC, IP, RC, RN, performed the experiment, AC, IP analyzed data, AC, IP, RC, wrote the paper, RC provided funds, VM provided instrumental support and technical expertise, all authors read, commented and finally agreed on the MS.

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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	0.90 ± 0.01	0.88	0.94
Dominant frequency (Hz)	48.10 ± 26.59	32	128	69.82 ± 27.29	64	192
Number of pulses	7.27 ± 4.26	2	31	11.74 ± 0.56	11	13
Duration of pulses (seconds)	0.03 ± 0.01	0.004	0.064	0.03 ± 0.08	0.015	0.071

Table S2 temporal and spectral parameters of AbW performed by foundresses and features of AbW playback track

Experimental apparatus

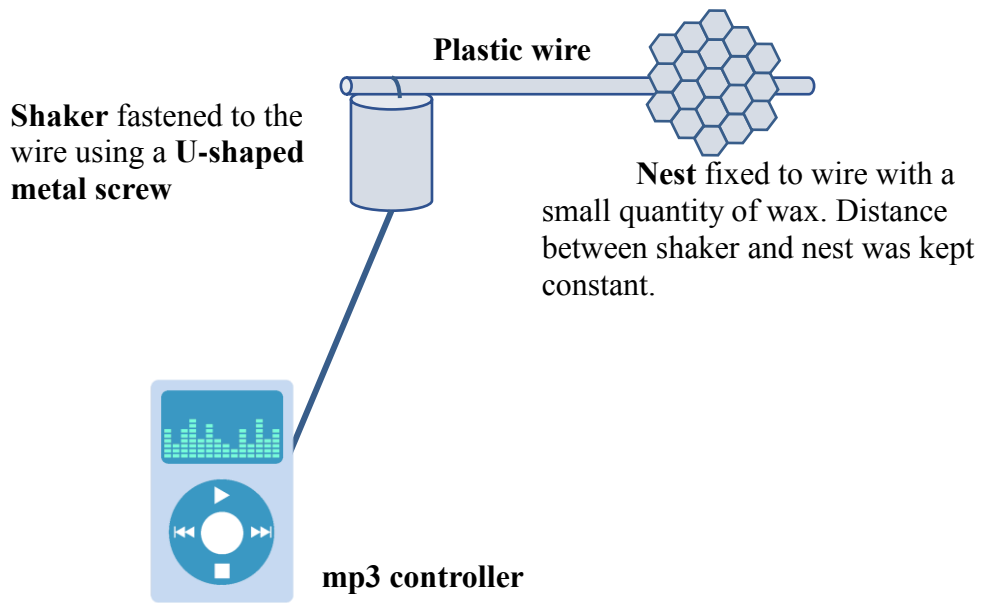


Figure S1 experimental apparatus of playback

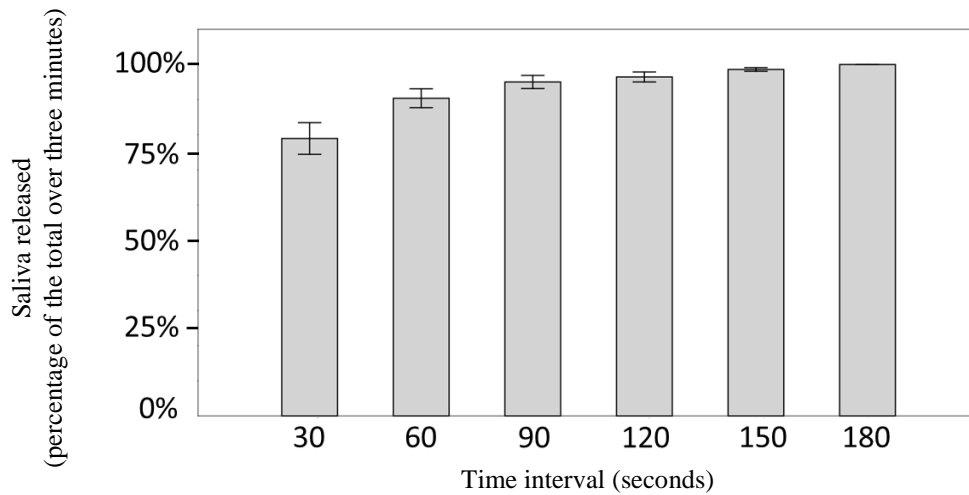


Figure S2 Percentage of saliva (mean \pm standard error) released in 30, 60, 90, 120, 150 and 180 seconds without vibrational stimulation.

Results

Source	Type III		
	Wald Chi-Square	df	Sig.
(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

Table S3 Influence of treatment, colony phase and larval size on binary logistic distribution of body movements. Results from Generalized Estimating Equation. Significant explanatory variables in bold.

Source	Type III		
	Wald Chi-Square	df	Sig.
(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 palpi movements. Results from Generalized estimating equation. Significant explanatory variables in bold.

Source	Type III		
	Wald Chi-Square	df	Sig.
(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 x Colony phase	0.071	2	0.965
Colony phase x Larval size	2.314	1	0.128
Treatment x Larval size	3.030	2	0.220

Table S5 Influence of treatment, colony phase and larval size on amount of larval saliva release in 30 seconds. Results from Generalized estimating equation. Significant explanatory variables in bold.

7. Discussion

In this thesis I investigated the use of visual and vibrational cues in some fundamental aspects of social insect life such as nestmate recognition, dominance hierarchies and adult-brood interactions. The role of these understudied sensory channels has been investigated in primitively eusocial paper wasp *P. dominula*, a model species for the study social communication and behavior (Turillazzi & West-Eberhard 1996, Pardi 1996, Dani 2006, Jeanne 2009, Bruschini et al. 2010, Jandt et al. 2014, Cervo et al. 2015).

The first part of this thesis (chapter 3) is focused on visual cues and on their involvement in nestmate recognition. Despite the ability in *P. dominula* to discriminate between nestmate and non-nestmate has been deeply studied and it's well known to be based on the perception of the chemical blend that covers the cuticle of each individual (Gamboa et al. 1986, Lorenzi et al. 1996, Gamboa 2004, Dani et al. 2001, Dani 2006, Bruschini et al. 2010), here, for the first time, it has been highlighted the importance of visual cues too. In particular, this study has shown that nestmate recognition based on visual cues (facial color pattern) acquires importance in early phase of colony cycle (before the emergence of workers) when only few females (foundresses) are present on the nest. Though chemical sensory channel could be reliable also in pre-emergence phase, visual cues might be advantageous over chemical ones because nestmate recognition based on facial pattern can be faster and does not require contact between individuals, so that the recognition could occur before or as soon as individuals land on the nest. A faster recognition of alien individuals approaching the nest by resident foundresses is probably a crucial ability to limit the numerous intraspecific usurpation attempts that each colony suffers in this phase of colony cycle (Cervo, 2006). As expected, instead, after the emergence of workers a major role in nestmate recognition is acquired by chemicals (Dani et al. 2001, Dani 2006). A chemical profile shared by all colony members (Gamboa 2004, Dani et al. 2001), indeed, allows a more reliable and accurate recognition respect to that based on visual cues. Facial pattern varies among a big number of colony members (thus requiring a high skill to learn and remember visual cues, Avarguès-Weber 2012) and at the same time could be the same among many individuals of the same population. However, the result of lack of different response towards discordant lures (i.e. lure composed of nest-mate visual cues and non-nestmate chemical cues vs lure that assembled non-nestmate visual cues with nestmate chemical cues), both in early as in late colony phase, suggests that both sets of cues influenced wasp behavior, despite alternatively each one of the two was not sufficient to allow NMR.

Overall, these results show the importance of visual cues for nestmate recognition in this species and suggest that visual and chemical cues interacted in multimodal and plastic

communication mode. In populations with a very low facial pattern variation and therefore where there may be colonies with all co-foundresses having no spotted clypeus (completely yellow clypeus) it will be needed to investigate on which cues nestmate recognition occurs. In such situations, it is possible that, in pre-emergence phase too, CHCs could assume a relevant role and may be more reliable than visual cues for an efficient nestmate recognition.

The second part of this thesis (chapter 4, 5 and 6) is focused on substrate-borne vibrational communication. For a long time, it has been hypothesized that the oscillatory behavior called abdominal wagging (AbW) performed by females on *P. dominula* nest could be performed to communicate among colony members (Pardi 1942, 1948). By studying vibrations produced by females in social context, namely in associative foundations of *P. dominula*, it has been possible to highlight that the position in the dominance hierarchy of each individual and its ovary development influence the intensity of vibrations (chapter 5). These results suggest that vibrations could bring information about individual fertility. Moreover, if this percussive behavior, as previously suggested (Hill 2009, Jeanne 2009), is strictly related to energetical costs for performing it, AbW could represent an honest quality signal. Since dominant position in colony hierarchy is related with more intense vibrations, AbW could be used by foundresses to maintain a dominant rank position and to suppress reproduction of subordinates. In this perspective AbW could be included in the ritualized dominance behavior pattern described by Pardi in 1940s and could be considered an extension of tactile and aggressive behaviors performed by dominant females towards subordinates (Pardi 1942, 1948). Manipulative and playback studies will be important to clarify the role of vibrations and their relative importance, in relation to behaviors and chemical cues, in hierarchy maintenance.

Moreover, the well-known host-parasite pair *P. dominula* – *P. sulcifer* has helped us to make progress in understanding the vibrations role in *Polistes* communication system (chapter 4). This obligate social parasite, indeed, needs to communicate both host larvae and adults the presence on the nest of a “super”- dominant female by inducing hosts to assume a worker/subordinate role and to rear parasite brood (Turillazzi et al. 1990, Cervo 2006). Vibrations recorded in usurped nests show that the parasite is able to exaggerate some parameters of host vibrational signals, i.e. intensity and duration. If host females use vibrations to maintain colony hierarchy, parasite could exploit this potential host vibrational communication system to impose herself as dominant individual among adult host females by exaggerating those vibrational parameters linked to social status. Although, in arthropods, temporal and spectral parameters of percussive signals seem to be related with morphological and physiological features of individual that produces these vibrations (Gibson & Uetz 2008, Hill 2008, 2009, Elias and Mason 2010), further studies to investigate

biological meaning of intensity and duration of vibrational events on *Polistes* nests are needed. Furthermore, the reported role of vibrations for *P. fuscatus* and *P. biglumis* suggests that the parasite can exploit *P. dominula* vibrational signals to drive larval development toward worker phenotype (Brillet 1999, Jeanne 2009, Suryanarayanan et al. 2011, Mignigni & Lorenzi 2015, Jandt 2017). Further playback studies will be necessary to evaluate the effects of vibrations and their parameters on *P. dominula* larval development as well as on the behavior and the physiology of adult females. Indeed, despite of only in few species the role of vibrations in inducing physiological change (Schneider et al. 2004, Suryanarayanan et al. 2011, Jandt et al. 2017), has been proved, no direct evidences have been reported so far in *P. dominula*.

Despite the results of chapters 4 and 5 indicate that vibrations could be signals involved in dominance communication, this does not exclude that AbW could also represent a signal with short-term effects involved in adult-larvae communications as proposed by Brennan (2007). The results demonstrate that vibrational stimuli played back on nest in absence of adults (therefore in absence of chemical and visual stimuli) do not induce or inhibit the release of salivary secretion by dismissing the *salivary release modulation hypothesis* proposed for this behaviour (Savoyard et al. 1998, Brennan 2007, Jeanne 2009). On the other hand, the same playback experiments furnish us the first experimental demonstration of vibrational communication between adult and larvae in *Polistes dominula*. In fact, AbW playback evokes a behavioral response of larvae by increasing their body movements (chapter 6). We can speculate that larvae implement their movements to signal their need to adults. Since AbW is performed during nest inspection and food distribution (Brillet et al. 1999, Brennan 2007), it is possible that hungry larvae move to attract adults attention and to request food. Further studies, in which AbW track will be played back on nests with hungry or satiated larvae, will clarify if AbW and larval behavioral response are involved in feeding communication.

Overall, the results of vibrational signals analysis indicate that AbW could be involved in social communication and could be part of dominance signalling system. Furthermore, like for other sensory channels (Dapporto et al. 2004, Lorenzi 2006, Ortolani et al. 2010), parasites could exploit *P. dominula* vibrational communication system in order to integrate themselves into host colonies and to successfully exploit the dominant positions in usurped host colonies. Although further studies are needed to clarify the biological meaning of host and parasite vibrations in adult-adult and adult-brood dominance interactions, an important result of this study is that specific substrate-borne vibrations produced by AbW are perceived by larvae and elicit a behavioural response by revealing that vibrations can convey an effective message in such context fitting the definition of biological communication (Scott-Phillips 2008).

By comparing results of vibrational analysis of two studies of this thesis (chapter 4 and chapter 5) there is an evident discrepancy in some vibrational parameters (dominant frequency and intensity). This is probably due to different conditions of the laboratories where the two studies were carried out i.e. table isolated from vibroacoustic environment for recordings trials of chapter 4 and normal table in a no-isolated laboratory for recordings trials of chapter 5. However, it is relevant to underline that this discrepancy does not affect the results obtained in the two studies because comparisons have been conducted within datasets obtained by recording vibrations with the same method and in the same laboratory conditions. Furthermore, the AbW track used in playback experiment has been selected from a set of vibrations recorded in the well isolated laboratory and under optimal vibroacoustical conditions to recording trials.

In conclusion, the results of this thesis, by using as model species the paper social wasp *P. dominula*, highlight that other communication channels apart from the chemical one can play an additional role in regulating fundamental aspects of insect social life. This study reveals an expected existence of a multimodal communication in *Polistes* wasps and underlines how different sensory channels can integrate or exchange in a complex communication system, by indicating that beyond chemicals social insect life are regulated by visual and vibrational signals.

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