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Rethinking recognition: social context in adult life rather than early experience shapes recognition in a social wasp

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Social recognition represents the foundation of social living. To what extent social recognition is hard-wired by early-life experience or flexible and influenced by social context of later life stages is a crucial question in animal behaviour studies. Social insects have represented classic models to investigate the subject, and the acknowledged idea is that relevant information to create the referent template for nest-mate recognition (NMR) is usually acquired during an early sensitive period in adult life. Experimental evidence, however, highlighted that other processes may also be at work in creating the template and that such a template may be updated during adult life according to social requirements. However, currently, we lack an ad hoc experiment testing the alternative hypotheses at the basis of NMR ontogeny in social insects. Thus, to investigate the mechanisms underlying the ontogeny of NMR in *Polistes* wasps, a model genus in recognition studies, and their different role in determining recognition abilities, we subjected *Polistes dominula* workers to different olfactory experiences in different phases of their life before inserting them into the social environment of a novel colony and testing them in recognition bioassays. Our results show that workers develop their NMR abilities based on their social context rather than through pre-imaginal and early learning or self-referencing. Our study demonstrates that the social context represents the major component shaping recognition abilities in a social wasp, therefore shedding new light on the ontogeny of recognition in paper wasps and prompting the reader to rethink about the traditional knowledge at the basis of the recognition in social insects.

This article is part of the theme issue 'Signal detection theory in recognition systems: from evolving models to experimental tests'.

1. Introduction

(a) Understanding the timing and cues of social recognition

Recognition represents the essential foundation of animal interactions. From sexual encounters between potential partners to the most complex animal societies, each individual has to recognize the ones encountered in order to decide how to behave towards them. Recognition occurs when specific phenotypic cues, or labels, expressed by an individual ('cue-bearer') are perceived by another individual ('evaluator') and then compared with an internal 'referent template' of the latter [1–4]. According to the degree of correspondence between the encountered phenotype labels and the referent template, the evaluator performs the consequent recognition action, ranging from strong rejection to complete acceptance [1–5]. Given the spread and importance of recognition processes, the mechanisms at their basis have been extensively studied over the years both in vertebrates and invertebrates [2–8].

In regard to the nature of the cues potentially used by animals to create their template and compare the phenotype of others, different sensory channels are often involved, depending on the species and the different recognition contexts [9–12]. Discrimination can occur through the use of environmental (e.g. nest

odour) or genetic cues: animals can learn their own phenotypes (self-referent phenotype matching) or those of their relatives, and later compare the phenotypes of unknown individuals with this learned recognition template [2–5,7,8,13].

Various processes explain when and how an animal acquires the crucial cues to create a template and perform correct phenotypic comparisons [2–5,14–17]. In particular, early experience coupled with a short sensitive period has been claimed to be a common and widespread evolutionary strategy to restrict the storage of biologically relevant information to a precocious developmental stage of the individual, thus reducing the risks of future recognition errors [15,17–21]. From the pioneer work of Lorenz [22] to the wide body of following research, early experience leading to imprinting-like phenomena appeared to play a major role in shaping recognition in animals [15,18–21,23–25]. Information acquired during an early sensitive window for learning is used by individuals across different contexts, to recognize parents and offspring [21,26,27], to discriminate kin from non-kin [28,29], to choose a sexual partner of the same species [24,30–33] and to select the most profitable food source [34–36]. In more recent years, however, growing attention has also been devoted to the importance of the social surroundings of an animal, especially for those species living in socially structured groups, for the development of efficient recognition systems, with different studies highlighting that in impoverished social circumstances individuals struggle to develop adequate recognition patterns [19,37–41]. In both vertebrates [37–39] and invertebrates [40,41], social isolation affects or impairs the individual's ability to recognize other group members.

A social group is usually a highly complex environment, which can change over time in both the composition and social role of their members, and recognition processes appear to be finely entwined with the social network of interactions that an individual experiences through its life [42,43]. Given the plasticity in the structure of many social environments [44–46], it may be evolutionarily advantageous that group members should reflect a certain degree of plasticity in their recognition system, with the ability to adjust or update their templates based on contextual contingencies [42,43,47]. For example, in many social insect species, the colony odour is dynamic and can change over time owing to multiple factors (i.e. colony age, usurpation or replacement of the dominant individuals, colony relocation to a new nesting site, social interactions and cue exchanges among colony members, the presence of parasites or pathogens, etc.) [48–56]; therefore, for the recognition system to maintain its efficacy, at least in some species, individuals should be able to update their template during their life [43,47]. In fact, experimental evidence [57–59] suggests that the sensitive early-life period might not be a shared feature in social species, opening the possibility that templates might be repeatedly updated during adult life according to social requirements [43,47,59,60]. Various species of ants showed a rather temporal flexible learning system for recognition cues [43,48,49,51] and also honeybees appear to readjust their recognition template after shifts in their colony odour [13,50,52].

In animal societies, a type of recognition essential to direct altruistic efforts towards the appropriate targets, maintain the group integrity and defend it from outer threats or exploitation is represented by the discrimination of group members with respect to alien conspecifics [61,62]. Nest-mate recognition (NMR) represents a remarkable process especially in social insects' colonies, where it is usually mediated by chemical cues (i.e. a blend of cuticular hydrocarbons (CHCs) covering the body surface of an individual) that are qualitatively similar in a species but can vary in their relative amounts among colonies of the same species [12,63]. In many social insect species, NMR is widely thought to be determined by early-life experiences [43,47]. However, previous research has demonstrated that other mechanisms also can shape NMR abilities in social insects [43,51,57–61] and, in particular, recent findings on *Polistes* paper wasps might overturn our current understanding of template formation in social wasps [59,64]. These studies have demonstrated that the social interactions and the environment experienced in adult life also can alter the individual NMR [40,41,51,53,59], challenging the common perspective of the acquisition of a rather rigid recognition template during a strict sensitive window [14,65]. At present, however, we lack an integrative study assessing the relevance of the different mechanisms (i.e. pre-imaginal learning, early experience, self-referencing, adult social context) at the basis of NMR in shaping recognition abilities in social insects.

(b) Paper wasps as model for the ontogeny of recognition processes

Paper wasps of the genus *Polistes* have represented for decades a model to study the ontogeny of NMR in social insects [7,14,66]. The acknowledged idea is that *Polistes* wasps learn the olfactory recognition cues from the paper of their natal nest during a short sensitive window, namely, the first few days (48–72 h) after emergence [7,14,66]. The nest material conveys the same chemicals as the colony inhabitants, providing information about colony membership [67], and it has therefore been considered the primary source of cues, necessary and sufficient, for the acquisition of the referent template [67]. Although it was generally believed that the NMR was shared within the *Polistes* genus, the ontogeny of such a process has been investigated in only a few of the 206 species of *Polistes* paper wasps, and recent studies, carried out on the European paper wasp *Polistes dominula* [47,59,64], have unveiled that the precocious sensitive window coupled with the nest paper as relevant cues do not represent the only mechanism to explain the development of recognition abilities in these wasps. In fact, these studies have already demonstrated that, at least in *P. dominula*, the template is formed neither during an early post-emergence window [59] nor during the pupal stage [64] and that the nest paper is not the most relevant source of cues for template formation [59]. Such experimental evidence opened new questions on the mechanisms underlying the ontogeny of NMR in *Polistes* wasps.

(c) Investigating recognition ontogeny through phase-dependent manipulation of olfactory experience

To investigate the mechanisms underlying the ontogeny of NMR in *Polistes* wasps, individuals in a specific stage of development can be subjected to differential odour experience experiments and, subsequently, to different social contexts, by introducing post-sensitive window adults into a novel social environment (i.e. nest). Individuals can then be tested in recognition bioassays recording the behavioural response (aggressive acts) towards different wasp lures (i.e. wasps freshly killed by freezing) (figure 1). Such an experimental design allows identification of the precise timing and the
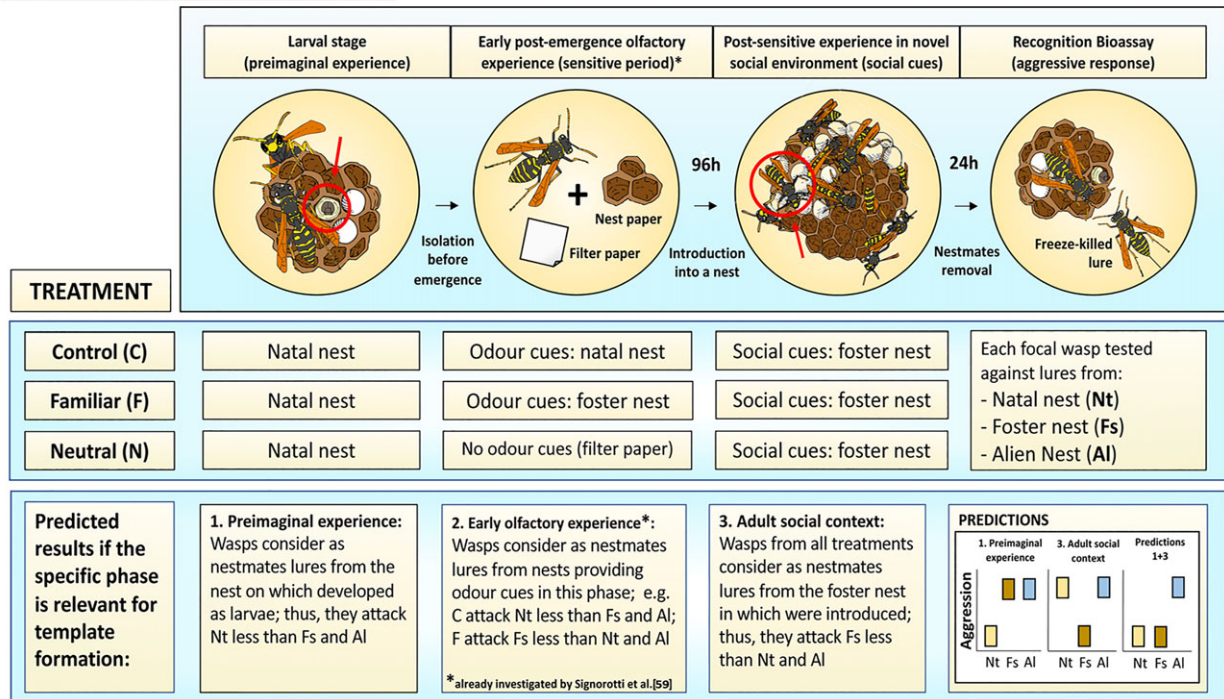
EXPERIMENTAL DESIGN

Figure 1. Experimental design used in the study. Wasps were subjected to different olfactory and social experiences in different phases of their life, from pre-imaginal (larval) to adult life and then tested in recognition biossays to assess which experience and phase was more relevant for the ontogeny of NMR abilities. *The effect of early post-emergence olfactory experience (prediction 2) had already been investigated by Signorotti et al. [59].

most relevant cues for template formation. For example, if the referent template is acquired as a result of olfactory experience during an early sensitive window post-emergence, it is possible to predict that, once tested in recognition bioassays, wasps will accept (i.e. lower aggressive response) lures bearing odour cues similar to those experienced by the wasp during the sensitive phase (figure 1,*). This protocol was adopted by Signorotti et al. [59], who removed pre-eclosing workers and pupae of *P. dominula* from their natal nests and exposed them to different odour experience (odour cues from natal/foreign nest, no odour cues) during an alleged sensitive window (pupal stage or first hours post-emergence) [14] before reintroducing them into their colony of origin. Once tested in recognition bioassays, wasps showed a higher acceptance of lures from their natal nests, regardless of their early olfactory experience [59]. Based on these results, we can hypothesize that the referent template of the tested wasps was acquired either before the pupal or early adult sensitive window, i.e. during the larval stage on the natal nest, or after, i.e. once reintroduced into their natal colony (figure 1), or through a different process of self-referent learning. Here, through a similar experiment of manipulation of early odour experience and of the social environment of adult wasps (introduction into a foster nest), we further investigate the mechanisms responsible for template formation in the same species, to finally assess the different importance of self-referencing, pre-imaginal or early olfactory experience, and social context in shaping the wasps' recognition abilities. In particular, our experimental design (figure 1) allows us to predict that (i) if the referent template is acquired during the larval stage or is based on a self-referencing process, wasps will be less aggressive towards nest-mate lures from their natal nest with respect to wasp lures from the foster colony or from an alien colony;

(ii) if the template is instead updated according to the olfactory and social experience during adult life in a biologically relevant context such as the foster nest, wasps should be less aggressive towards foster nest-mate lures than towards wasp lures from their natal nest or from an alien colony; and (iii) finally, if all the different processes (pre-imaginal larval experience, self-referencing and template update due to social context) play a role in shaping recognition abilities, experimental wasps should be less aggressive toward both the foster nest-mate and natal nest-mate lures while attacking the alien wasp lure never encountered before.

The main aim of the present study is to identify the differential contribution of the processes involved in template formation in *P. dominula* to challenge the traditional view on the ontogeny of NMR abilities in social wasps.

2. Material and methods

(a) Collection and laboratory rearing

Colonies of *P. dominula* ($n = 60$) in which the first generation of workers had already eclosed were collected in late June 2015 in three different localities, at least 9 km apart to avoid any relatedness among tested wasps, throughout Tuscany (Italy). All nests were in a comparable stage of development with approximately 80 cells and contained immature brood (i.e. eggs, larvae and pupae). Colonies were transferred in glass boxes ($15 \times 15 \times 15$ cm) and provided with sugar, fly maggots and water ad libitum. Boxes were kept in the laboratory under natural photoperiod at *ca* 25°C. Wasps on the nests at collection were identified as foundresses or workers [68] and marked on the wings with different acrylic colours (Testor Enamel) to distinguish among them and from newly emerged workers. Four days after collection, when marked adults were at least 3 days old, a

time window essential to allow the development of a complete cuticular chemical profile in this wasps [69], five marked workers were removed from each nest and killed by freezing to be used later as lures.

To identify and remove pre-eclosing wasps from their natal nest at the end of their pupal stage, to ensure that they were not exposed to their colony odour or social environment in the early phase of their adult life, we used the same protocol adopted by Signorotti et al. [59]. Cell caps were partially removed with clean forceps before wasps' emergence and the colour and movements of pre-eclosing workers were observed. Developing pupae with both bright yellow/black colours and moving heads and antennae were selected as pre-eclosing workers according to [59].

(b) Experimental design

Pre-eclosing workers were gently removed from their cells with soft tweezers and individually transferred into plastic Petri dishes (2×1.5 cm). Workers were divided into three groups, which experienced a different odour exposure during the early hours of their adult life: (i) 21 wasps (C = control) were transferred to Petri dishes containing natal nest material (corresponding to about three empty cells) (odour cues) and a piece of filter paper (2×1 cm); (ii) 21 wasps (F = familiarized) were transferred to Petri dishes containing a comparable amount of nest material (odour cues) from a foreign nest of a different population and a piece of filter paper (2×1 cm); and (iii) 22 wasps (N = neutral) were transferred to Petri dishes containing a piece of filter paper (2.5 cm^2) to control for the effect of the paper material without odour cues. For all treatments, filter paper was previously washed with pentane for 15 min to remove any contamination. Thus, the odour cues were present exclusively on the nest paper inside the Petri dishes in treatments C and F. Washed filter paper was added as a neutral control for the paper material and to have a similar amount of paper in the three different treatments. Petri dishes were provided with a hole for air and a small bee-candy (68% sucrose, 32% glucose–fructose syrup) as food for the wasp. To ensure that the sensitive phase for learning (a few hours after emergence) reported for other Polistes species [7,66] was included in our experimental temporal window, wasps were left in Petri dishes for 4 days. To investigate the effect of the social contexts after the early olfactory experience, the post-sensitive window adult wasps were introduced into a novel social environment (i.e. foster nest). Each wasp was then individually marked and transferred to a foreign nest (foster nest) from a different population before being tested in recognition bioassays recording the behavioural response (aggressive acts) towards different wasp lures the following day, to allow acclimatization [59]. F workers were transferred to the nests that had provided the nest fragments for the early exposure in Petri dishes. Nests were maintained at 8°C in a refrigerator for 1 h before introducing the focal wasp in order to calm down the resident wasps and facilitate acceptance of focal wasps by foster nest-mates. Nests were monitored for 1 h after introduction of focal wasps to evaluate the behavioural response of resident wasps towards the foreign individual. Regardless of treatment, focal wasps approaching nests were inspected by resident wasps with their antennae, but no particular aggressive reaction was recorded towards the introduced individuals. After acceptance, experimental wasps were left for 24 h on their foster colonies before recognition bioassays were performed. Signorotti et al. [59] showed that a time window of 24 h on a nest is sufficient for the focal wasps to develop their NMR abilities and to perform the typical behavioural repertoire towards approaching intruders (presented lures) [70–72]. Another four wasps belonging to the three different treatments (1 C, 1 F, 2 N) did not approach the nest during the hour post-introduction into the cage and

were not on the nest the following day; thus, they were not tested in recognition bioassays.

(c) Recognition bioassays

Before starting the bioassays, all resident wasps except the experimental individual were removed from each nest to avoid any interference from resident wasps [59]. Each experimental wasp was left undisturbed on its nest for at least 15 min. A total of 60 experimental wasps (20 C, 20 N and 20 F) were tested. To evaluate the recognition abilities of experimental individuals, we presented each wasp with three different kinds of lures and we recorded their behavioural responses [59]. Lures were represented by a worker from the natal nest, a worker from the foster nest and an alien worker from a foreign colony of a different population. Lures were thawed to room temperature before trials. During bioassays, each lure was held with forceps and slowly introduced into the cage containing the experimental wasp on its adoptive nest. The lure was held about 1 cm from the nest and maintained for 1 min after the first contact between the focal wasp and the lure (bite or antennal inspection). Aggression toward a lure could depend in part on how threatening the lure is perceived to be because of both its size and appearance. Each triplet of lures was thus created using wasps that had comparable dimensions, measured as maximum head width under a Wild M5A stereomicroscope (difference in head width among lures of each triplet was less than 1.17%) [73]. All the lures were mounted on the forceps with the same posture, by holding the lure legs with the forceps. The antennae of the lures were open wide in the same position with the help of forceps and the stick bearing each lure was introduced into the cage containing the nest from the side opposite to the nest and slowly brought near the nest so that the focal wasp would see the face of the approaching lure. The three lures were presented to experimental wasps in a random order with a 30 min interval between presentations. Each lure was used only once. Experimenters performing and video-recording lure presentations were blind to lures' identities. Videos were blind-watched by a third observer. The number of bites given to each lure by the focal wasp was counted for statistical analysis. Bites are the most evident and quantifiable aggressive behaviour performed by wasps during this kind of experiment and aggressiveness towards wasp lures represents a good proxy of NMR since alien intruders are bitterly attacked and repelled, while nest-mates are inspected through antennation and peacefully ignored [59,70,71]. The general level of aggression among the treatment groups might inform about the motivation of wasps to defend the colony depending on their different early olfactory experience. Furthermore, as we provided contrasting olfactory versus social cues, our experimental design also allows us to infer to what degree NMR is impaired by contrasting information during individual ontogeny. In fact, the combination of different early olfactory experience with social environment may have a modulatory or synergic effect on the behavioural response of the wasps. For example, C wasps, which experienced olfactory cues from their natal nest during the early adult sensitive phase and contrasting social cues from the foster nest, might respond less aggressively to lures from both the natal and foster nests with respect to aliens. Similarly, 'F' wasps, which experienced concordant early olfactory and social cues from the foster nest, could defend more strenuously their adoptive colony and be more aggressive towards aliens with respect to 'C' and 'N' wasps.

Finally, to verify if the introduction into a novel social environment (foster nest), not bearing any cues from the natal colony of the tested wasps, might alter their behavioural response, for example by reducing the motivation of the wasp to defend a foreign unrelated nest, we compared the level of aggression towards wasp lures recorded in our experiment

with the aggressive response showed by wasps in the previous work using a similar experimental design [59]. In the study by Signorotti *et al*. [59], wasps were subjected to the same protocol of early olfactory experience, with the only difference that they were reintroduced into their own natal nests instead of a foster nest. The comparisons of the results from the present study with those from [59] should highlight the focal wasps' ability to recognize their true colony of origin after being introduced on a nest. If focal wasps recognize their colony of origin from the foster colony as a result of cues learned through self-referencing or during the larval stage, we could expect a different behavioural response in the two experiments, in terms of both intensity and differential treatment of the presented lures. In fact, wasps could more intensively defend their natal colony to which they are related, while being less motivated in the defence of the foster colony.

(d) Statistical analyses

We used generalized estimating equations (GEEs) to assess the importance of lure category (natal nest, foster nest, alien), treatment category (control, neutral or familiarized) and their interaction on aggressive response (i.e. number of bites given to each lure), and we set colony of origin for each focal wasp as subject effect to control for non-independence of focal wasp from the same nests (range of number of cases per subject was from 3 to 18). We used Poisson distribution with log-link function, and an independent working correlation matrix structure. To calculate effect size we computed Cohen's d as $d = (m_a - m_b)/$s.d. where m_a and m_b are the estimated marginal means of each category within the pairwise comparison, and s.d. is the pooled standard deviation, which is calculated as

$$\text{s.d.} = \sqrt{\frac{(N_a - 1)(\text{s.d.}_a)^2 + (N_b - 1)(\text{s.d.}_b)^2}{N_a + N_b - 2}},$$

where N_a, N_b, s.d.$_a$ and s.d.$_b$ are, respectively, sample size and standard deviation of each category [74]. The interpretation of d is as follows: small effect: $d = 0.2$, medium effect: $d = 0.5$, large effect: $d = 0.8$ [75]. The non-parametric Mann–Whitney test was used to compare the aggressive response (number of bites) towards lures from the natal nests, alien lures and lures from the colony into which the wasps were reintroduced in the two experiments. All the statistical analyses were carried out using IBM SPSS 20.0.

3. Results

We found a significant effect of lure category (Wald $\chi^2 = 61.949$, d.f. = 2, $p < 0.001$) and no effect of treatment category (Wald $\chi^2 = 0.238$, d.f. = 2, $p = 0.888$) on the focal wasp aggressive response. Indeed, the same pattern of NMR was evident for the three treatments (no significant interaction between lure category and treatment category: Wald $\chi^2 = 3.079$, d.f. = 4, $p = 0.545$): lures from the foster nests were less attacked than aliens and lures from the natal nests, which were both attacked to a similar extent (table 1). On average, lures from the foster nests received approximately six times fewer bites than lures from the natal nests or aliens across the three treatments (respectively, 6.18 ± 0.12 and 5.80 ± 1.59, figure 2). In all cases, the effect size was similar ($d \approx 0.2$, table 1), indicating that the pattern of NMR response (aggression) was similar across treatments.

In regard to the comparison with the results from the previous study [59], focal wasps did not show a significant difference in the aggressive response towards alien

Table 1. Results from GEEs show that in all treatments focal wasps were less aggressive towards the foster nest lure (FS) than towards natal nest (NT) and alien (AL) lures, which were attacked at a comparable rate (see the electronic supplementary material). n.a., not applicable.

treatment	comparison	average fold contrast in aggression[a]	*p*-value	effect size
control	NT versus FS	4.800	0.033	0.190
	AL versus FS	6.182	0.001	0.139
	AL versus NT	1.288	0.996	n.a.
neutral	NT versus FS	4.968	<0.001	0.190
	AL versus FS	6.307	<0.001	0.203
	AL versus NT	1.270	0.771	n.a.
familiarized	NT versus FS	7.640	<0.001	0.277
	AL versus FS	6.060	<0.001	0.209
	AL versus NT	0.793	0.998	n.a.

[a]Aggression corresponds to the number of bites received by each lure.

lures in the two experiments (Mann–Whitney U-test, $Z = 1.693$, $p = 0.091$) (figure 3), whereas they responded differently to the lures from the natal nests (Mann–Whitney U-test, $Z = -6.298$, $p < 0.0001$), which were fiercely attacked in our bioassays while being accepted in the previous experiment [59] (figure 3). Most interestingly, lures from the foster nests of our experiment were treated as lures from the natal nests in the earlier study (Mann–Whitney U-test, $Z = -0.646$, $p = 0.477$, figure 3).

4. Discussion

Our results show that, in the social paper wasp *P. dominula*, template formation or update occurs during adult life, regardless of self-referencing and previous pre-imaginal or early post-imaginal olfactory experiences and actual nestmateship. Experimental wasps responded to different lures as if the lure from the foster colony was recognized as nestmate, while the lure from the natal nest and the alien lure were equally treated as foreign individuals and strenuously attacked to defend the adoptive nest. The wasps' inability to recognize lures from their actual colony of origin suggests that our workers did not rely either on self-referent, pre-imaginal or early olfactory cues [3,4,14]; otherwise we could have expected a lower aggressive response towards lures from the natal nest [14,59,61]. Such inability is unlikely to be due to lack or insufficiency of relevant cues, since workers had sufficient time to acquire olfactory cues in the alleged early sensitive period [7,14,66,69] before being introduced into their foster colony. Instead, focal wasps strenuously defended their adoptive nests against both natal nest and alien lures, highlighting that they considered the foster colonies as their true colonies of origin. If workers were able to use self-referent cues or to discriminate those from their natal colony, we might have expected them to put little effort into defence of the foster nest against intruders, especially towards individuals from their colony of origin, but this does not seem the case for our wasps.
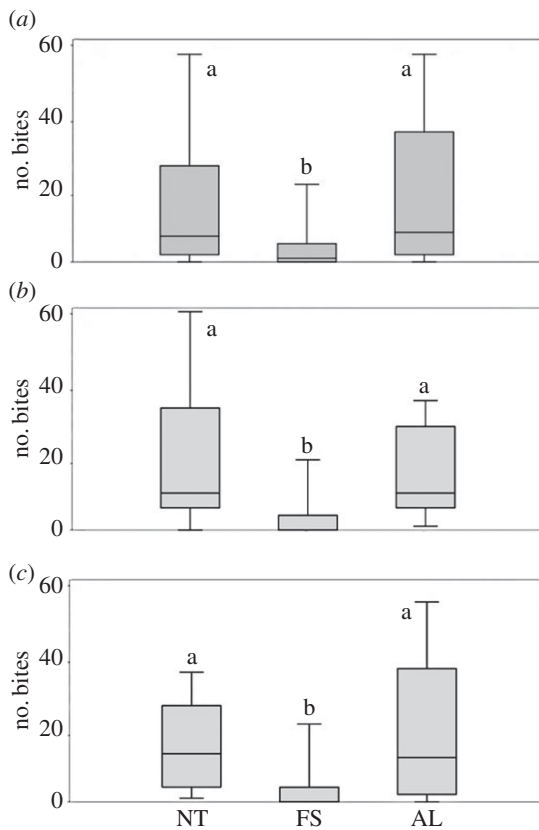
Figure 2. Aggressive responses (number of bites) of experimental wasps towards wasp lures belonging to the three different categories (natal nest (NT), foster nest (FS), alien (AL)) for the three experimental treatments: (*a*) 'control', (*b*) 'familiarized' and (*c*) 'neutral'. Thick horizontal lines represent medians, boxes show upper and lower quartiles and whiskers indicate the highest and lowest values.
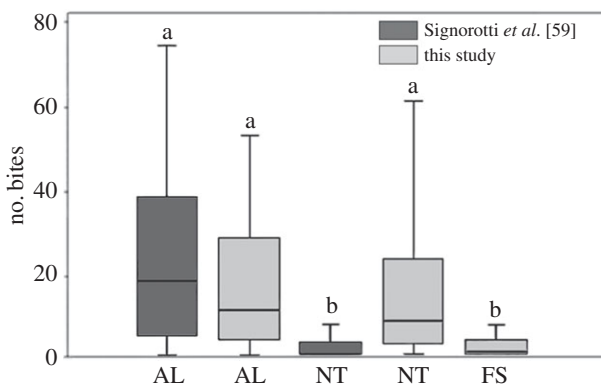

Figure 3. Comparison of the aggressive responses (number of bites) of experimental wasps from the present study and from Signorotti *et al.* [59] towards alien lures (AL), lures from the natal nest (NT) and foster nest (FS) lures. Thick horizontal lines represent medians, boxes are upper and lower quartiles and whiskers indicate the highest and lowest values.

The comparison of our results with those of the previous study by Signorotti *et al.* [59] highlighted a similar pattern of aggressive response of focal wasps towards alien wasp lures, which means that they were equally motivated to defend the nest in both the experimental contexts. Instead, the degree of aggressiveness towards lures from the natal nests greatly varied between the two experiments, as they were accepted by wasps in [59] and bitterly repelled, similarly to alien intruders, in the present experiment. Such a result, suggesting that

focal wasps were unable to recognize nest-mates from their actual colony of origin, becomes even more interesting when considering the fact that our lures from the foster colonies were treated as lures from the natal nest in [59], since both were accepted by focal wasps. The indiscriminate acceptance of lures from the colonies into which experimental wasps were introduced (natal nest in [59], foster colony in the present study) in both experiments suggests that workers recognized as nest-mates the lures coming from the first nest that they encountered during adult life. It would be possible to argue that the major role of the social context represented by a nest with its inhabitants in shaping NMR abilities with respect to an early olfactory experience may be simply due to the different extents of the chemical stimuli represented by a fully developed colony versus a limited amount of nest paper. However, the previous literature on NMR in *Polistes* wasps [7,14,66], from which the model for the ontogeny of NMR recognition in social wasps has been drawn, demonstrated that *Polistes* wasps belonging to different species were able to acquire the necessary cues for developing a NMR template even after being exposed for a very short time (1 or 2 h) to nest fragments [66], while exposure to adult nest-mates outside the social environment of the colony did not allow the development of correct NMR abilities [76]. Our results may appear in contrast with these earlier studies that investigated the ontogeny of recognition abilities in *Polistes* wasps, demonstrating that exposure solely to the nest paper during the first few hours post-emergence was necessary and sufficient to form a reliable referent template [7,14,66,76]. Nevertheless, previous research on the ontogeny of NMR was carried out using other temperate species of *Polistes* as model species [7,66,76], and it is possible that different species might evolve different strategies, involving also different sensory channels, to recognize potential intruders [72,77]. Moreover, these studies [7,66], as well as most of the pioneering researches exploring the ontogeny of recognition abilities in both vertebrates and invertebrates, tested the existence of a critical window for cue-learning and template formation in experimental conditions of social deprivation [7,37–41,66]. Individuals were isolated for a period of time during different developmental phases, presented with specific cues and then tested in subsequent bioassays for their recognition abilities without being exposed to biologically relevant social milieux [7,37–41,66].

The behavioural response showed by our *Polistes* workers looks similar to the behaviour of ant workers kidnapped by slave-making species. Slave-workers are kidnapped by slave-raiding ants at the pupal stage and eclose in the parasite nest, towards which they develop a social attachment, joining in all aspects of colony labour [78]. It is generally thought for social insects that after eclosion individuals should imprint to the odour of their nest, adult nest-mates and brood in any colony in which they eclose [78,79]. In most cases, this will be their natal colony, and all adult nest-mates and brood will be more or less closely related. Thus, it should be adaptive for a worker to defend and take care of those individuals encountered in the nest where it encloses [61,63,79]. Different species of social parasites of the genus *Polistes* appear to exploit such a process by usurping host colonies and modifying their chemical profile during the pre-emergence phase before eclosion of the first generation of workers [80]. Social parasites benefit from this peculiarity since host workers eclosing from the raided brood or in the usurped colony imprint to the present social environment

and are manipulated to work for individuals to which they are not related [78–80].

Despite the potential exploitation from social parasites, updating the referent template in adult life may be beneficial for maintaining an effective NMR system since the odour of individuals, and consequently the odour of the colony they inhabit, can change over time [48,49,52,53]. This is especially true for those species of social Hymenoptera where colony members continually exchange recognition cues and need frequent social interactions with nest-mates to maintain an accurate referent template reflecting the current shared odour of the colony, as demonstrated in several ant species [51,60]. Template updating has also been shown in the honey-bee, *Apis mellifera*, where guard workers at the colony entrance change their referent template after a comb's transfer between hives, with an increase in acceptance of non-nest-mates belonging to the 'comb donor' hive [52]. The transfer does not change the bees' individual odour, but guards update their colony odour template directly from the wax comb rather than from the contact with other conspecifics [52]. In social wasps, no direct evidence for a template updating are available so far [47], but, once again, studies on social parasitism in *Polistes* wasps have indirectly indicated a similar process of updating. For instance, *Polistes biglumis* workers recognize only the odour of their species in non-parasitized colonies, but learn a template that fits the odour of their own species and that of the social parasite *Polistes atrimandibularis* after colony usurpation [80]. Although exploited by social parasites, the ability to retard, adjust or update the referent template may still be advantageous in *Polistes* annual societies, where female functional caste is relatively labile and change at the top of the hierarchy, with subsequent variation of the colony odour, is likely, especially in those species, such as *P. dominula*, characterized

by associative founding, with several reproductive females, sometimes unrelated, cooperating to build a new colony at the beginning of the season but still competing over dominance [81,82].

Overall, our study demonstrates, in a social wasp, that the social context experienced in adult life can represent the major component for the formation of the NMR template, stronger than genetic cues of nest-mateship or self-referencing and regardless of previous pre-imaginal or early post-imaginal olfactory experiences during time-restricted sensitive phases. We believe that our results prompt the reader to rethink about the traditional knowledge at the basis of the ontogeny of NMR in social insects, highlighting a previously unreported plasticity in the development of recognition abilities in insects, which are often considered as simple and stereotyped organisms, and opening new avenues for research.

References

1. Reeve HK. 1989 The evolution of conspecific acceptance thresholds. *Am. Nat.* **133**, 407–435. (doi:10.1086/284926)

2. Sherman PW, Reeve HK, Pfennig DW. 1997 Recognition systems. In *Behavioural ecology: an evolutionary approach* (eds JR Krebs, NB Davies), pp. 69–96. Oxford, UK: Blackwell Scientific Publications.

3. Mateo JM. 2004 Recognition systems and biological organization: the perception component of social recognition. *Ann. Zool. Fenn.* **41**, 729–745.

4. Tsutsui ND. 2004 Scents of self: the expression component of self/non-self recognition systems. *Ann. Zool. Fenn.* **41**, 713–727.

5. Liebert AE, Starks PT. 2004 The action component of recognition systems: a focus on the response. *Ann. Zool. Fenn.* **41**, 747–764.

6. Stuart RJ. 1992 Nestmate recognition and the ontogeny of acceptability in the ant, *Leptothorax curvispinosus*. *Behav. Ecol. Sociobiol.* **30**, 403–408. (doi:10.1007/BF00176175)

7. Gamboa GJ, Reeve HK, Ferguson ID, Wacker TL. 1986 Nestmate recognition in social wasps: the origin and acquisition of recognition odours. *Anim.*

Behav. **34**, 685–695. (doi:10.1016/S0003-3472(86)80053-7)

8. Mateo JM. 2010 Self-referent phenotype matching and long-term maintenance of kin recognition. *Anim. Behav.* **80**, 929–935. (doi:10.1016/j.anbehav.2010.08.019)

9. Endler JA. 1993 Some general comments on the evolution and design of animal communication systems. *Phil. Trans. R. Soc. Lond. B* **340**, 215–225. (doi:10.1098/rstb.1993.0060)

10. Brennan PA, Kendrick KM. 2006. Mammalian social odours: attraction and individual recognition. *Phil. Trans. R. Soc. B* **361**, 2061–2078. (doi:10.1098/rstb.2006.1931)

11. Brumm H, Naguib M. 2009 Environmental acoustics and the evolution of bird song. *Adv. Stud. Behav.* **40**, 1–33. (doi:10.1016/S0065-3454(09)40001-9)

12. Blomquist GJ, Bagnères AG. 2010 *Insect hydrocarbons: biology, biochemistry, and chemical ecology*. Cambridge, UK: Cambridge University Press.

13. Breed MD, Leger EA, Pearce AN, Wang YJ. 1998 Comb wax effects on the ontogeny of honey bee nestmate recognition. *Anim. Behav.* **55**, 13–20. (doi:10.1006/anbe.1997.0581)

14. Gamboa GJ, Reeve HK, Pfennig DW. 1986 The evolution and ontogeny of nestmate recognition in social wasps. *Annu. Rev. Entomol.* **31**, 431–454. (doi:10.1146/annurev.en.31.010186.002243)

15. Caspers BA, Hoffman JI, Kohlmeier P, Krüger O, Krause ET. 2013 Olfactory imprinting as a mechanism for nest odour recognition in zebra finches. *Anim. Behav.* **86**, 85–90. (doi:10.1016/j.anbehav.2013.04.015)

16. Bos N, Grinsted L, Holman L. 2011 Wax on, wax off: nest soil facilitates indirect transfer of recognition cues between ant nestmates. *PLoS ONE* **6**, e19435. (doi:10.1371/journal.pone.0019435)

17. Fénéron R, Jaisson P. 1995 Ontogeny of nestmate brood recognition in a primitive ant, *Ectatomma tuberculatum* Olivier (Ponerinae). *Anim. Behav.* **50**, 9–14. (doi:10.1006/anbe.1995.0215)

18. Bolhuis JJ, Honey RC. 1998 Imprinting, learning and development: from behaviour to brain and back. *Trends Neurosci.* **21**, 306–311. (doi:10.1016/S0166-2236(98)01258-2)

19. Champalbert A, Lachaud JP. 1990 Existence of a sensitive period during the ontogenesis of social

behaviour in a primitive ant. *Anim. Behav.* **39**, 850–859. (doi:10.1016/S0003-3472(05)80949-2)

20. Knudsen EI. 2004 Sensitive periods in the development of the brain and behavior. *J. Cogn. Neurosci.* **16**, 1412–1425. (doi:10.1162/0898929042304796)

21. Sluckin W. 2017 *Imprinting and early learning*. New York, NY: Routledge.

22. Lorenz K. 1937 The companion in the bird's world. *Auk* **54**, 245–273. (doi:10.2307/4078077)

23. Kruijt JP. 1962 Imprinting in relation to drive interactions in Burmese red junglefowl. *Symp. Zool. Soc. Lond.* **8**, 219–226.

24. Immelmann K. 1972 Sexual and other long-term aspects of imprinting in birds and other species. *Adv. Stud. Behav.* **4**, 147–174. (doi:10.1016/S0065-3454(08)60009-1)

25. Errard C, Le Guisquet AM, Christidès JP, Mercier JL, Lenoir A, Hefetz A. 2008 Early learning of volatile chemical cues leads to interspecific recognition between two ant species. *Insect. Soc.* **55**, 115–122. (doi:10.1007/s00040-008-0979-4)

26. Beecher MD. 1981 Development of parent-offspring recognition in birds. In *Development of perception* vol. 1 (eds RK Aslin, JR Alberts, MR Petersen), pp. 45–66. New York, NY: Academic Press.

27. Beecher MD. 1991 Successes and failures of parent-offspring recognition in animals. In *Kin recognition* (ed. PG Hepper), pp. 94–124. Cambridge, UK: Cambridge University Press.

28. Hepper PG. 1986 Kin recognition: functions and mechanisms a review. *Biol. Rev.* **61**, 63–93. (doi:10.1111/j.1469-185X.1986.tb00427.x)

29. Gerlach G, Hodgins-Davis A, Avolio C, Schunter C. 2008 Kin recognition in zebrafish: a 24-hour window for olfactory imprinting. *Proc. R. Soc. B* **275**, 2165–2170. (doi:10.1098/rspb.2008.0647)

30. Irwin DE, Price T. 1999 Sexual imprinting, learning and speciation. *Heredity* **82**, 347–354. (doi:10.1038/sj.hdy.6885270)

31. Slagsvold T, Hansen BT, Johannessen LE, Lifjeld JT. 2002 Mate choice and imprinting in birds studied by cross-fostering in the wild. *Proc. R. Soc. Lond. B* **269**, 1449–1455. (doi:10.1098/rspb.2002.2045)

32. Verzijden MN, ten Cate C. 2007 Early learning influences species assortative mating preferences in Lake Victoria cichlid fish. *Biol. Lett.* **3**, 134–136. (doi:10.1098/rsbl.2006.0601)

33. Verzijden MN, ten Cate C, Servedio MR, Kozak GM, Boughman JW, Svensson EI. 2012 The impact of learning on sexual selection and speciation. *Trends Ecol. Evol.* **27**, 511–519. (doi:10.1016/j.tree.2012.05.007)

34. Burghardt GM, Hess EH. 1966 Food imprinting in the snapping turtle, *Chelydra serpentina*. *Science* **151**, 108–109. (doi:10.1126/science.151.3706.108)

35. Punzo F. 2002 Food imprinting and subsequent prey preference in the lynx spider, *Oxyopes salticus* (Araneae: Oxyopidae). *Behav. Process.* **58**, 177–181. (doi:10.1016/S0376-6357(02)00031-1)

36. Darmaillacq AS, Chichery R, Dickel L. 2006 Food imprinting, new evidence from the cuttlefish *Sepia officinalis*. *Biol. Lett.* **2**, 345–347. (doi:10.1098/rsbl.2006.0477)

37. Harlow HF, Harlow MK. 1962 Social deprivation in monkeys. *Scient. Am.* **207**, 137–146. (doi:10.1038/scientificamerican1162-136)

38. Kruijt JP. 1971 Early experience and the development of social behaviour in junglefowl. *Psychiat. Neurol. Neurochir.* **74**, 7–20.

39. Groothuis T, Van Mulekom L. 1991 The influence of social experience on the ontogenetic change in the relation between aggression, fear and display behaviour in black-headed gulls. *Anim. Behav.* **42**, 873–881. (doi:10.1016/S0003-3472(05)80139-3)

40. Boulay R, Lenoir A. 2001 Social isolation of mature workers affects nestmate recognition in the ant *Camponotus fellah*. *Behav. Process.* **55**, 67–73. (doi:10.1016/S0376-6357(01)00163-2)

41. Lenoir A, Cuisset D, Hefetz A. 2001 Effects of social isolation on hydrocarbon pattern and nestmate recognition in the ant *Aphaenogaster senilis* (Hymenoptera, Formicidae). *Insect. Soc.* **48**, 101–109. (doi:10.1007/PL00001751)

42. Breed MD, Buchwald R. 2009 Cue diversity and social recognition. In *Organization of insect societies. From genome to sociocomplexity* (eds J Gadau, J Fewell), pp. 173–193. Cambridge, MA: Harvard University Press.

43. Bos N, d'Ettorre P. 2012 Recognition of social identity in ants. *Front. Psychol.* **3**, 83.

44. Schradin C. 2013 Intraspecific variation in social organization by genetic variation, developmental plasticity, social flexibility or entirely extrinsic factors. *Phil. Trans. R. Soc. B* **368**, 20120346. (doi:10.1098/rstb.2012.0346)

45. Faulkes CG, Bennett NC. 2013 Plasticity and constraints on social evolution in African mole-rats: ultimate and proximate factors. *Phil. Trans. R. Soc. B* **368**, 20120347. (doi:10.1098/rstb.2012.0347)

46. Henzi SP, Forshaw N, Boner R, Barrett L, Lusseau D. 2013 Scalar social dynamics in female vervet monkey cohorts. *Phil. Trans. R. Soc. B* **368**, 20120351. (doi:10.1098/rstb.2012.0351)

47. Signorotti L, Cervo R, d'Ettorre P. 2015 Ontogeny of nestmate recognition in social Hymenoptera. In *Social recognition in invertebrates* (eds L Aquiloni, E Tricarico), pp. 165–191. Berlin, Germany: Springer.

48. VanderMeer RK, Saliwanchik D, Lavine B. 1989 Temporal changes in colony cuticular hydrocarbon patterns of *Solenopsis invicta*: implications for nestmate recognition. *J. Chem. Ecol.* **15**, 2115–2125. (doi:10.1007/BF01207442)

49. Provost E, Rivière G, Roux M, Morgan ED, Bagnères AG. 1993 Change in the chemical signature of the ant *Leptothorax lichtensteini* Bondroit with time. *Insect Biochem. Mol. Biol.* **23**, 945–957. (doi:10.1016/0965-1748(93)90112-6)

50. Breed MD, Garry MF, Pearce AN, Hibbard BE, Bjostad LB, Page Jr RE. 1995 The role of wax comb in honey bee nestmate recognition. *Anim. Behav.* **50**, 489–496. (doi:10.1006/anbe.1995.0263)

51. Boulay R, Hefetz A, Soroker V, Lenoir A. 2000 *Camponotus fellah* colony integration: worker individuality necessitates frequent hydrocarbon exchanges. *Anim. Behav.* **59**, 1127–1133. (doi:10.1006/anbe.2000.1408)

52. Couvillon MJ, Caple JP, Endsor SL, Kärcher M, Russell TE, Storey DE, Ratnieks FL. 2007 Nest-mate recognition template of guard honeybees (*Apis mellifera*) is modified by wax comb transfer. *Biol. Lett.* **3**, 228–230. (doi:10.1098/rsbl.2006.0612)

53. Newey PS, Robson SKA, Crozier RH. 2009 Temporal variation in recognition cues: implications for the social life of weaver ants *Oecophylla smaragdina*. *Anim. Behav.* **77**, 481–488. (doi:10.1016/j.anbehav.2008.11.003)

54. van Zweden JS, Dreier S, d'Ettorre P. 2009 Disentangling environmental and heritable nestmate recognition cues in a carpenter ant. *J. Insect Physiol.* **55**, 158–163. (doi:10.1016/j.jinsphys.2008.11.001)

55. Cappa F, Bruschini C, Protti I, Turillazzi S, Cervo R. 2016 Bee guards detect foreign foragers with cuticular chemical profiles altered by phoretic varroa mites. *J. Apic. Res.* **55**, 268–277. (doi:10.1080/00218839.2016.1229886)

56. Cervo R, Bruschini C, Cappa F, Meconcelli S, Pieraccini G, Pradella D, Turillazzi S. 2014 High *Varroa* mite abundance influences chemical profiles of worker bees and mite–host preferences. *J. Exp. Biol.* **217**, 2998–3001. (doi:10.1242/jeb.099978)

57. Liu Z, Yamane S, Wang Q, Yamamoto H. 1998 Nestmate recognition and temporal modulation in the patterns of cuticular hydrocarbons in natural colonies of Japanese carpenter ant *Camponotus japonicus* Mayr (Hymenoptera: Formicidae). *J. Ethol.* **16**, 57–65. (doi:10.1007/BF02769283)

58. Stroeymeyt N, Guerrieri FJ, van Zweden JS, d'Ettorre P. 2010 Rapid decision-making with side-specific perceptual discrimination in ants. *PLoS ONE* **5**, e12377. (doi:10.1371/journal.pone.0012377)

59. Signorotti L, Cappa F, d'Ettorre P, Cervo R. 2014 Novel insights into the ontogeny of nestmate recognition in *Polistes* social wasps. *PloS ONE* **9**, e97024. (doi:10.1371/journal.pone.0097024)

60. van Zweden JS, Brask JB, Christensen JH, Boomsma JJ, Linksvayer TA, d'Ettorre P. 2010 Blending of heritable recognition cues among ant nestmates creates distinct colony gestalt odours but prevents within-colony nepotism. *J. Evol. Biol.* **23**, 1498–1508. (doi:10.1111/j.1420-9101.2010.02020.x)

61. d'Ettorre P, Lenoir A. 2010 Nestmate recognition. In *Ant ecology* (eds L Lach, C Parr, K Abbott), pp. 194–208. New York, NY: Oxford University Press.

62. Breed MD. 2014 Kin and nestmate recognition: the influence of WD Hamilton on 50 years of research. *Anim. Behav.* **92**, 271–279. (doi:10.1016/j.anbehav.2014.02.030)

63. van Zweden JS, d'Ettorre P. 2010 Nestmate recognition in social insects and the role of hydrocarbons. In *Insect hydrocarbons: biology, biochemistry and chemical ecology* (eds GJ Blomquist, AG Bagnères), pp. 222–243. Cambridge, UK: Cambridge University Press.

64. Signorotti L, d'Ettorre P, Sguanci G, Cervo R. 2015 Pupal experience and nestmate recognition in *Polistes dominula* wasps. *Insect. Soc.* **62**, 433–441. (doi:10.1007/s00040-015-0422-6)

65. Crozier RH, Pamilo P. 1996 *Evolution of social insect colonies*. Oxford, UK: Oxford University Press.

66. Pfennig DW, Gamboa GJ, Reeve HK, Reeve JS, Ferguson ID. 1983 The mechanism of nestmate discrimination in social wasps (*Polistes*, Hymenoptera: Vespidae). *Behav. Ecol. Sociobiol.* **13**, 299–305. (doi:10.1007/BF00299677)

67. Espelie KE, Hermann HR. 1990 Surface lipid of the social wasp *Polistes annularis* (L.) and its nest and nest pedicel. *J. Chem. Ecol.* **16**, 1841–1852. (doi:10.1007/BF01020498)

68. West-Eberhard MJ. 1969 The social biology of polistine wasps. *Misc. Publ. Mus. Zool. Univ. Mich.* no. 140.

69. Lorenzi MC, Sledge MF, Laiolo P, Sturlini E, Turillazzi S. 2004 Cuticular hydrocarbon dynamics in young adult *Polistes dominulus* (Hymenoptera: Vespidae) and the role of linear hydrocarbons in nestmate recognition systems. *J. Insect Physiol.* **50**, 935–941. (doi:10.1016/j.jinsphys.2004.07.005)

70. Ortolani I, Zechini L, Turillazzi S, Cervo R. 2010 Recognition of a paper wasp social parasite by its host: evidence for a visual signal reducing host aggressiveness. *Anim. Behav.* **80**, 683–688. (doi:10.1016/j.anbehav.2010.07.003)

71. Cappa F, Beani L, Cervo R. 2016 The importance of being yellow: visual over chemical cues in gender recognition in a social wasp. *Behav. Ecol.* **27**, 1182–1189. (doi:10.1093/beheco/arw025)

72. Cini A, Cappa F, Pepiciello I, Platania L, Dapporto L, Cervo R. 2019 Sight in a clique, scent in society: plasticity in the use of nestmate recognition cues along colony development in the social wasp *Polistes dominula*. *Front. Ecol. Evol.* **7**, 444. (doi:10.3389/fevo.2019.00444)

73. Eickwort KR. 1969 Differential variation of males and females in *Polistes exclamans*. *Evolution* **23**, 391–405. (doi:10.1111/j.1558-5646.1969.tb03523.x)

74. Coe R. 2002 It's the effect size, stupid. What effect size is and why it is important. In *Annu. Conf. Br. Edu. Res. Ass., 12–14 September 2002, Exeter, UK*. See http://www.leeds.ac.uk/educol/documents/00002182.htm.

75. Cohen J. 2013 *Statistical power analysis for the behavioral sciences*. New York, NY: Routledge.

76. Shellman JS, Gamboa GJ. 1982 Nestmate discrimination in social wasps: the role of exposure to nest and nestmates (*Polistes fuscatus*, Hymenoptera: Vespidae). *Behav. Ecol. Sociobiol.* **11**, 51–53. (doi:10.1007/BF00297666)

77. Cervo R, Cini A, Turillazzi S. 2015 Visual recognition in social wasps. In *Social recognition in invertebrates* (eds L Aquiloni, E Tricarico), pp. 125–145. Cham, Switzerland: Springer.

78. Lenoir A, d'Ettorre P, Errard C, Hefetz A. 2001 Chemical ecology and social parasitism in ants. *Annu. Rev. Entomol.* **46**, 573–599. (doi:10.1146/annurev.ento.46.1.573)

79. Cini A, Sumner S, Cervo R. 2019 Inquiline social parasites as tools to unlock the secrets of insect sociality. *Phil. Trans. R. Soc.* **374**, 20180193. (doi:10.1098/rstb.2018.0193)

80. Lorenzi MC, Cometto I, Marchisio G. 1999 Species and colony components in the recognition odor of young social wasps: their expression and learning (*Polistes biglumis* and *P. atrimandibularis*; Hymenoptera: Vespidae). *J. Insect Behav.* **12**, 147–158. (doi:10.1023/A:1020906631121)

81. Queller DC, Zacchi F, Cervo R, Turillazzi S, Henshaw MT, Santorelli LA, Strassmann JE. 2000 Unrelated helpers in a social insect. *Nature* **405**, 784. (doi:10.1038/35015552)

82. Leadbeater E, Carruthers JM, Green JP, Rosser NS, Field J. 2011 Nest inheritance is the missing source of direct fitness in a primitively eusocial insect. *Science* **333**, 874–876. (doi:10.1126/science.1205140)

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9