

Original Article

Available kin recognition cues may explain why wasp behavior reflects relatedness to nest mates

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Relatedness is predicted to be a key determinant of cooperative behavior, but kin discrimination within social insect colonies is surprisingly rare. A lack of reliable cuticular hydrocarbon (CHC) cues is thought to be responsible, but here we show that in a high-profile paper wasp model, kin recognition cues are available for some individuals that found nests with nonrelatives. Thus, unrelated *Polistes dominulus* helpers could potentially recognize themselves as such. On this basis, we reanalyzed a behavioral data set to investigate whether foraging effort, defense contributions and aggression toward nest mates might thus reflect CHC profiles. Both foraging behavior and aggression varied with genetic relatedness, but genetic relatedness itself was a better predictor of this variation than differences in CHC profiles. We propose that wasps use specific components of the CHC profile, the identity of which is as yet unknown, to identify relatives among nest mates. Our data provide the first evidence of within-nest kin discrimination in primitively eusocial wasps but leave open the question of which cues are responsible.

Key words: direct fitness, kin discrimination, *Polistes*, primitively eusocial wasps, relatedness, social behavior.

INTRODUCTION

Within cooperatively breeding groups, inclusive fitness theory predicts that individuals should preferentially cooperate with their closest relatives (Hamilton 1964). In vertebrates, “helper” individuals often modify their helping behavior according to their relatedness to nest mates (Griffin and West 2003; Cornwallis et al. 2009; Zottl et al. 2013), but within-nest kin discrimination is rare in social insects (Gamboa 1996; Keller 1997; Boomsma and d’Ettorre 2013). Although some individuals often contribute less to group productivity than others (Cant et al. 2006a, 2006b; Field et al. 2006), there is little evidence that these individuals are the most distantly related to the breeder. This is widely thought to reflect mechanistic constraints because social insect kin discrimination is typically based on cuticular hydrocarbon (CHC) profiles (Gamboa 1996; d’Ettorre and Lenoir 2010), which usually become mixed among nest mates to create a *Gestalt* odor representing the group as a whole (Crozier

and Dix 1979; van Zweden et al. 2010). Mixing maximizes the potential for recognition of non-nest mates, but limits within-nest kin recognition (d’Ettorre and Lenoir 2010; van Zweden et al. 2010; Boomsma and d’Ettorre 2013).

In the paper wasp *Polistes dominulus*, individuals often cofound nests with complete nonrelatives (Queller et al. 2000; Zanette and Field 2008). Nests are built in the spring by cofoundress groups of 1–20 wasps, where 1 individual typically monopolizes reproduction and her subordinates forage to raise the brood, defend the nest from predators and social parasites, and perform other brood-care activities (Pardi 1942). There are 2 reasons why unrelated cofoundresses may have CHC profiles that are recognizably different from those of their nest mates. First, although variation in relatedness is common in social insect nests, the presence of nonrelatives is not (Queller et al. 2000), and it may be that mixing of hydrocarbon cues does not fully eliminate a genetic signature in species such as *P. dominulus*. Indeed, previous work has suggested that such cues may well be available, at least when wasps first emerge after the pupal stage (Dani et al. 2004). Second, although turnover of dominants means that genetically unrelated wasps can emerge from the

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same natal nests, at least some unrelated subordinates are “interlopers” from other natal nests (Queller et al. 2000; Leadbeater E, Dapporto L, personal observation). These individuals are particularly likely to have CHC profiles that are recognizably different from that of their cofoundresses.

In this study, we begin by testing whether foundresses could potentially use CHC profiles to discriminate kin from nonkin during the spring brood-rearing period. We predict that differences in CHC profiles between pairs of nest mates, quantified as “CHC-distance”, will correlate with genetic relatedness. We then reanalyze previous data to establish whether differences in CHC profiles might explain variation in subordinate social behavior, including foraging effort, nest defense, and aggression toward nest mates. For an unrelated subordinate, the only fitness benefits of group membership derive from the possibility of inheriting the nest, should the dominant die (Queller et al. 2000), or through laying occasional eggs while still subordinate (Leadbeater et al. 2011). These possibilities are sufficient to make joining a group of nonrelatives a better option than nesting alone (Leadbeater et al. 2011). For relatives of the dominant, nest inheritance is also possible, but all is not lost if it does not occur. If taking risks (e.g., by foraging) increases the dominant’s productivity, a subordinate that is related to the dominant should be more willing to risk mortality (Kokko and Johnstone 1999; Cant and Field 2001, 2005; Cant et al. 2006b). Even if she dies, her contribution will not have been fruitless because her cofoundresses can successfully rear the dominant’s brood to adulthood (Gadagkar 1990; Shreeves et al. 2003). In contrast, an unrelated subordinate that dies without reproducing directly has no kin-selected interest in the brood that will be raised without her, so any investment that she has made in her potential future workforce will be entirely redundant.

Theory, thus, predicts that unrelated subordinates should adaptively tailor their behavior to minimize mortality risk, by contributing relatively less to risky helping tasks such as foraging and nest defense (Cant and Field 2001). When the opportunity to inherit the nest finally arises, unrelated subordinates should also compete more aggressively for the dominant position (Cant et al. 2006a, 2006b). Yet, empirical studies have never found any such differences (Cant and Field 2001; Cant et al. 2006a; Leadbeater et al. 2010), although Queller et al. (2000) present data that suggest nonrelatives may forage less. We proposed that using the kin recognition cues that are available to wasps themselves, rather than genetic relatedness, might prove a more sensitive test to detect correlations with behavior. By doing so, we can take into account that some nonrelatives might not recognize themselves as such. We predict that differences in CHC profiles between pairs of nest mates, quantified as CHC-distance, will correlate with genetic relatedness and will be a better predictor of behavior than genetic relatedness itself.

METHODS

In early spring 2009, we searched for newly built *P. dominulus* nests on cactus hedges at a low-intensity arable site near Conil de la Frontera, southern Spain. All foundresses ($n = 129$ from 28 nests, group size range: 2–7) were individually paint-marked during predawn collections, and a tarsal sample was taken for genotyping. These data represent a subset of the nests described in Leadbeater et al. (2010). For 6 weeks after group stabilization but prior to the emergence of the first offspring, we randomly selected groups of nests from our sample, and sampled CHC profiles by gently

rubbing a piece of filter paper on each wasp’s thoracic scutum for 30 s using sterile forceps (Turillazzi et al. 1998; Dapporto et al. 2005). Each sample was wrapped in an individual aluminum sheet and then stored in a sterile vial. Behavioral observations began on the day following CHC sampling for each nest.

Behavioral data

We identified the dominant wasp on each nest by daytime censuses (Cant and Field 2005). Each nest was visited approximately every 45 min on sunny afternoons for 4 days, noting the presence or absence of group members (mean \pm SD: 28 ± 7 surveys per nest in total). Dominants rarely leave the nest (Pardi 1942; Cant and Field 2005), so following previous authors (Cant and Field 2001; Cant et al. 2006a; Zanette and Field 2009), we identified the dominant wasp as the individual that was found on the nest most often. If there were 2 wasps that were consistently present on the nest after 4 days of censusing (<3 census visits difference; e.g., 1 wasp might be present for 20 census visits, and another for 18), we continued to census for another day until the dominant was clearly identified. Previous work has confirmed that this technique reliably identifies the dominant wasp on *P. dominulus* nests via microsatellite genotyping of pupae (Leadbeater et al. 2011).

We then carried out 3 behavioral assays, previously described (Leadbeater et al. 2010) and detailed again below.

Foraging effort

Since mortality when foraging is high (Cant and Field 2001), we predicted a negative correlation between CHC distance to the dominant and foraging effort. Foraging effort was estimated as the proportion of surveys in which each subordinate was away from the nest during the first 4 days of censusing. Individuals that were not present at early morning (0530–0700 h) censuses immediately prior to or following surveying were excluded from the analysis because their absence was unlikely to be due to foraging. This produced a sample size of 75 subordinate wasps from 28 nests.

Nest defense

We carried out nest defense assays on one of the 4 afternoons when we assayed foraging effort, after censusing had finished for the day. We filmed the reaction of each nest’s cofoundresses to a dead conspecific “usurper” (from a distant site, killed by freezing) held with clean forceps, approximately 1 cm from the nest, for 2 min. Subordinates’ behavior was classed as “aggressive” if they performed one or more aggressive acts (Cant et al. 2006a) toward the usurper, including “lunges” (leaping across nest, physical contact), “chews” (light biting), “grapples” (physical grasping), and “mounts” (climbing onto a nest mate). Assays were carried out when the temperature was still high enough to allow active nest defense ($>$ approximately 20 °C), but this meant that some subordinates were not present because they were foraging. These individuals were excluded from the analysis, producing a sample size of 36 individuals from 23 nests.

Aggression when competing for the dominant position

We hypothesized that subordinates that can perceive themselves as unrelated may compete more aggressively for the dominant position when it becomes vacant. On the first day of sunny weather after the foraging survey phase of our experiment had been completed, we removed the dominant during a predawn census visit and subsequently video recorded the nest for 4 h to monitor aggressive interactions between subordinate cofoundresses. For each wasp,

we calculated the mean number of aggressive acts initiated and received per hour during the film, based on aggression categories listed above. We analyzed data for all subordinates that were present at the morning census when the dominant was removed and were present on the nest for more than 30 min during the filming period ($n = 47$ wasps from 17 nests).

After our behavioral assays were completed, we continued day-time censusing as described in *Foraging effort* to establish the inheritance rank of each individual, because inheritance rank has been previously shown to influence behavior in primitively eusocial wasps (Cant and Field 2001; Cant et al. 2006b; Field et al. 2006). Once the dominant of each nest had been removed (*Aggression when competing for the dominant position*), we continued to census that nest until we could identify the next wasp to become dominant. We then removed that wasp, and repeated the process until all inheritance ranks were known. This procedure has been previously described in Zanette and Field (2009) and requires at least 4 days of sunny weather censuses per wasp. Since mortality rates were high, we could establish the inheritance ranks of 90 wasps (62 subordinates) from our sample.

CHC analysis

Epicuticular compounds were extracted from the filter paper in 300 μL of pentane for 10 min. The solution was dried in a nitrogen stream and reelected in 25 μL of heptane for gas chromatograph–mass spectrometry analysis. We injected 2 μL of the solutions into a Hewlett Packard (Palo Alto, California) 5890A gas chromatograph coupled with an HP 5971A mass selective detector. A fused silica capillary column coated with 5% diphenyl and 95% dimethyl polysiloxane (ZB-5, 30 m \times 0.25 mm \times 0.1 μm ; Zebron) was used in the gas chromatography. The injector port and transfer line were set at 300 $^{\circ}\text{C}$, and the carrier gas was helium (at 12 psi). The temperature protocol was 70–150 $^{\circ}\text{C}$ at a rate of 12 $^{\circ}\text{C}/\text{min}$ (held for 2 min) and 150–320 $^{\circ}\text{C}$ at 8 $^{\circ}\text{C}/\text{min}$ (held for 5.08 min). Analyses were performed in splitless mode. Identification of cuticular compounds was performed on the basis of their mass spectra produced by electron impact ionization (70 eV). Peak areas of the epicuticular gas chromatogram of each wasp were transformed into percentages of the total CHCs.

CHC profiles were calculated based on the 50 most common hydrocarbons detected in more than 75% of individuals. CHC distance between pairs of individuals was the Euclidean distance between arcsine-transformed CHC profiles. Several alkenes and long linear alkanes have been found to reflect dominant status in this species (Sledge et al. 2001, 2004; Dapporto et al. 2007); linear alkanes have also previously been found to contribute little to kin discrimination, at least between non-nest mates (Dani et al. 2001). We removed these compounds from CHC profiles and repeated our analyses. All results were unchanged whether or not compounds that have been identified as important in the dominance signal in this species were included in CHC profiles.

Relatedness

To ascertain genetic relatedness between each subordinate and the dominant wasp on her nest, tarsal samples were genotyped at 8 microsatellite loci (Pdom1, Pdom2, Pdom7, Pdom20, Pdom25, Pdom127b, Pdom140, and Pbel28TAG) using standard methods that have previously been described for this sample in Leadbeater et al. (2010).

Statistical analyses

We performed 2 sets of statistical analyses. Analysis A aimed to establish whether CHC profile dissimilarity between pairs of wasps reflected genetic relatedness within nests. Analysis B focused on whether CHC distance was a better predictor of behavior than genetic relatedness. In each case, we used an information theoretic approach, comparing candidate models and selecting the best according to Akaike's information criterion (AIC; Burham and Anderson 2002).

For Analysis A, we first tested whether genetic relatedness between pairs of wasps correlated with CHC distance across the whole population, using a Mantel test (R package *ecodist*; Goslee and Urban 2007). Then, to focus specifically on kin recognition between nest mates, we compared a series of general linear mixed effects models (R package *lme4*; Bates et al. 2011) in which CHC distance between pairs of nest mates was the response variable. A Mantel test is not suitable for this analysis because separate tests would be needed for each nest. Instead, we randomly selected 1 wasp as the focal individual for each nest, specifying the CHC distances between that wasp and each nest mate as the response variable. For example, for Nest 1, wasp A might be the focal individual, and distances AB, AC, and AD would be included in the analysis, with focal wasp specified as a random factor. We analyzed 79 nest-mate pairs. To ensure that the random sample we tested was representative of the population, we performed a post hoc sensitivity analysis, randomly reallocating the focal wasp in each nest to create 999 parallel data sets for comparison.

Our basic model contained only the constant, the residual variance, and focal wasp as a random factor. We compared this with 5 candidate models containing combinations of additional fixed factors (Table 1): Genetic relatedness, sampling date (since profiles might become more similar as the season progresses and wasps spend more time together on a nest), and body size difference between the wasp pair. We identified the model with the lowest AIC value as the best fit to the data. Because some candidate models had AIC values within 2 units of the best model, we created a 95% confidence set and averaged these to estimate effect sizes and standard errors based on Akaike weights (Johnson and Omland 2004).

Analysis B aimed to establish whether CHC distance was a better predictor of subordinate behavior than genetic relatedness. First, we focused on subordinate foraging effort. Our basic model contained the intercept and residual variance, plus nest, sampling date, and group size as random factors. The proportion of surveys in which the subordinate was absent was the response variable (binomial error distribution). We compared this with 7 candidate models containing combinations of additional fixed effects (Table 2a): Genetic relatedness to the dominant, CHC distance from the dominant, and individual body size. The best model was identified based on the lowest AIC value. Note that our candidate models did not include inheritance rank as a predictor because inheritance ranks are not independent of one another and because mortality during the census period meant that we attained inheritance ranks for only a subset of subordinates. We investigated the relationship between inheritance rank, body size, genetic relatedness, and CHC distance for this subset based on Pearson's r .

Exactly the same procedure was followed to investigate aggression when the opportunity to inherit arose (response: number of aggressive interactions initiated by individual subordinates, factored by the time that they were present during the film), although we focused on relatedness/CHC distance to subordinate nest mates,

Table 1
Candidate models to predict CHC distance between pairs of nest-mate wasps (Analysis A, see details in Methods)

Model	AIC	Δ AIC	Akaike weight (W_i)	% Support in sensitivity analysis
Basic	-78.68	5.77	0.02	9.4
Relatedness*	-82.98	1.47	0.21	42.7
Size difference*	-82.43	2.03	0.16	12.5
Sampling date	-76.69	7.77	0.01	0.9
Relatedness + size difference*	-84.35	0	0.44	31.2
Relatedness + size difference + interaction*	-82.46	2.00	0.16	3.3

The basic model contained only the intercept and focal wasp as a random factor. All other models contained the basic model and the additional fixed factors specified. The best model (based on lowest AIC value) is indicated in bold. Parameter estimates were based on model averaging of all models in the 95% confidence set. The final column indicates the percentage of iterations where the model in question emerged as the best model in our sensitivity analysis.

* Indicates that the model was included in the 95% confidence set, from which fixed parameters were estimated.

rather than to the dominant wasp, following the logic that competitive interactions in this context do not involve the formerly dominant wasp (Table 2b). To investigate participation in nest defense, we compared the basic model with models containing single fixed factors only: CHC distance to the dominant, relatedness to the dominant, or body size because of our limited sample size for this data set ($n = 36$, Table 2c).

RESULTS

Analysis A

Across our entire sample, genetic relatedness was significantly negatively correlated with CHC distance (Figure 1; Mantel's $r = -0.05 \pm 0.021$ [97.5% confidence interval], $P < 0.01$). The best model to predict CHC distance between nest mates was relatedness + size difference, providing a significant improvement on the basic model (Δ AIC for basic model = 5.77; Table 1). Closely related nest-mate pairs had smaller CHC distances (Figure 1; estimate = -0.10 , 95% confidence interval [CI]: -0.16 to -0.04 ; estimates are based on model-averaged parameters from the 95% confidence set). Nest-mate pairs that were similar in size also had smaller CHC distances (estimate = 0.06 , 95% CI = 0.02 – 0.10). We found no correlation between size difference and relatedness (Pearson's $r = -0.14$, $P = 0.2$), and the interaction effect between size difference and relatedness was not statistically significant (estimate = -0.001 , 95% CI = -0.03 to 0.13). In our sensitivity analysis, one of the 4 models in our 95% confidence set emerged as the best model to predict the data in 89.7% of permutations, with relatedness attaining the most support overall (42% of permutations; Table 1). Including an interaction between body size and relatedness improved the model on only 3.3% of iterations.

Analysis B

The best model to predict foraging effort included individual body size (estimate: 0.14 ± 0.18 [standard error, SE]), relatedness to the dominant (estimate: 16.82 ± 3.38 [SE]), and their interaction (estimate: -1.46 ± 0.29 [SE], Table 2a, Figure 2). For wasps that were close relatives of the dominant, foraging effort was negatively correlated with body size, but for more distant relatives, the correlation was close to zero (Figure 3a). This effect of body size might reflect an underlying effect of inheritance rank because smaller wasps tended to occupy significantly lower ranks (Pearson's $r = -0.32$, $P = 0.01$; Figure 4).

Table 2a

Candidate models to predict subordinate behavior (Analysis B, see details in Methods): Foraging effort

	AIC	Δ AIC
Basic	325.82	51.91
Body size	298.57	24.66
Relatedness to dominant wasp	323.04	49.13
CHC distance to dominant wasp	318.06	44.15
Body size + relatedness to dominant wasp	297.76	23.85
Body size + CHC distance to dominant wasp	299.54	25.63
Body size + Relatedness to dominant wasp + interaction	273.91	0
Body size + CHC distance to dominant wasp + interaction	291.23	17.32

In all cases, the basic model contained only the intercept and focal wasp, group size, and sampling date as random factors. All other models contained the basic model and the additional fixed factors specified. In each case, a binomial error structure was specified. The model that was finally selected is indicated in bold.

Table 2b

Candidate models to predict subordinate behavior (Analysis B, see details in Methods): Aggression when opportunities to inherit arose

	Initiation rate		Receiving rate	
	AIC	Δ AIC	AIC	Δ AIC
Basic	135.80	16.50	134.12	1.04
Body size	129.59	10.29	134.31	1.22
Mean relatedness to nest mates	137.50	18.19	135.93	2.85
Mean CHC distance to nest mates	137.79	18.49	133.08	0
Body size + mean relatedness to nest mates	131.28	11.98	136.20	3.12
Body size + mean CHC distance to nest mates	131.59	12.29	133.96	0.87
Body size + mean relatedness to nest mates + interaction	119.31	0	136.41	3.33
Body size + mean CHC distance to nest mates + interaction	133.52	14.21	134.84	1.76

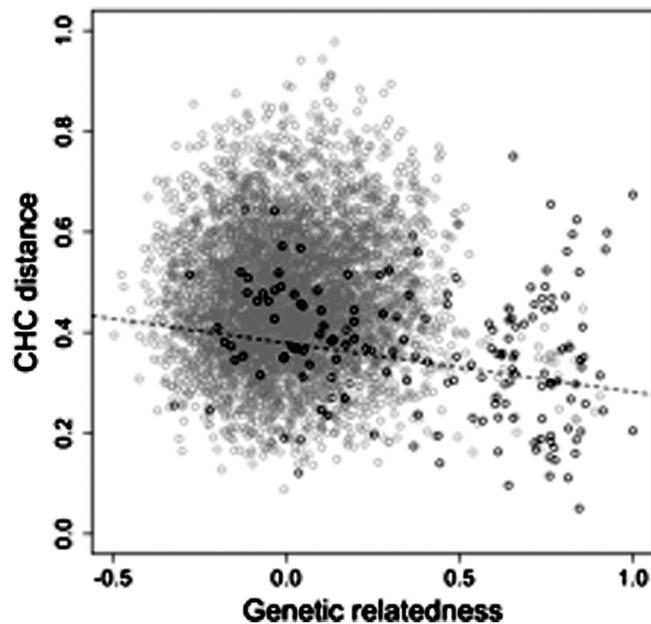
In all cases, the basic model contained only the intercept and focal wasp, group size, and sampling date as random factors. All other models contained the basic model and the additional fixed factors specified. In each case, a binomial error structure was specified.

Table 2c

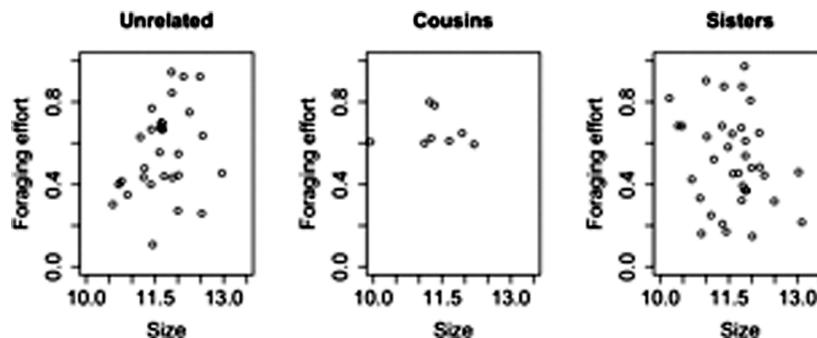
Candidate models to predict subordinate behavior (Analysis B, see details in Methods): Participation in nest defense

	AIC	Δ AIC
Basic	43.45	2.49
Body size	40.96	0
Relatedness to dominant	43.16	2.20
CHC distance to dominant	43.63	2.67

In all cases, the basic model contained only the intercept and focal wasp, group size, and sampling date as random factors. All other models contained the basic model and the additional fixed factors specified. In each case, a binomial error structure was specified. The model that was finally selected is indicated in bold.

**Figure 1**

The relationship between CHC distances between pairs of wasps and genetic relatedness. Points in gray derive from non-nest-mate pairs, and points in black come from nest mates. The dashed line represents parameter estimates for nest-mate pairs, based on the best model identified in Analysis A (see details in Methods).

**Figure 2**

Foraging effort was negatively correlated with body size (an indicator of inheritance rank) for wasps that were closely related to the dominant wasp. For those individuals that were less closely related, body size was positively correlated with foraging effort—smaller wasps foraged less. For presentation purposes, wasps have been classified as unrelated subordinates, cousins, or sisters of the dominant using the program Kingroup (Konovalov et al. 2004).

Aggression toward nest mates when the opportunity to inherit arose was best predicted by a model containing individual body size (estimate: 1.73 ± 0.38 [SE]), mean relatedness to nest mates (estimate: 40.48 ± 10.08 [SE]), and a 2-way interaction (estimate: -3.42 ± 0.86 [SE]). For close relatives of the dominant, the correlation between aggression and body size was negative but close to zero, but for distant relatives, the correlation was positive (Figure 3b). We found no similar effect for the rate at which aggression was received. Although the best model to predict receiving rate included CHC distance, this did not represent a significant improvement on the basic model (Δ AIC for basic model < 2). Finally, nest defense participation was best predicted by body size; smaller wasps were more aggressive (estimate: -2.59 ± 1.38 [SE]), but note that models including both relatedness and size difference were not included in the analysis because of limited sample size.

DISCUSSION

Social insect colonies where sufficient CHC profile variation exists for sister groups to be reliably identified are rare in the literature (Keller 1997; van Zweden et al. 2010, 2011; but see Page et al. 1991; Arnold et al. 1996; Arnold et al. 2000; Bonckaert et al. 2011; Nehring et al. 2011). Uniform colony odors may be selectively advantageous because they facilitate nest-mate recognition, particularly in large groups (Boomsma and d'Ettore 2013). Here, however, we find that CHC profiles vary sufficiently within nest-mate groups to potentially allow for kin discrimination between cofoundresses of the primitively eusocial wasp *P. dominulus*. But why might primitively eusocial groups differ from other social insects in this respect? One possibility is that homogenized colony odors are less important in small groups (Boomsma and d'Ettore 2013), though there is little evidence for reliable CHC variation between nest mates in other species with small colony sizes (Soro et al. 2011). Alternatively, our findings might reflect the semisocial life history of polistine cofoundresses because cofounding groups build new nests, rather than remaining as a family unit on their mother's nest, and are known to cofound with individuals from different natal nests (Queller et al. 2000).

Although CHC distance correlated with genetic relatedness, and thus might provide a cue by which unrelated subordinates could recognize themselves as such, CHC distance was a worse predictor of behavior than genetic relatedness. Two possible explanations

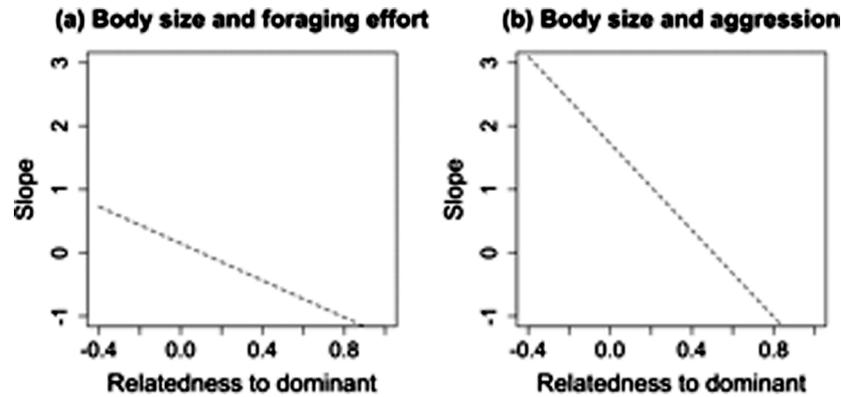


Figure 3

Predicted effect of relatedness on the slope of the relationship between (a) body size and foraging effort and (b) body size and aggression. Slopes are based on parameter estimates from the best-fitting models. Predicted relatedness between sisters is 0.75 and between nonrelatives is 0.

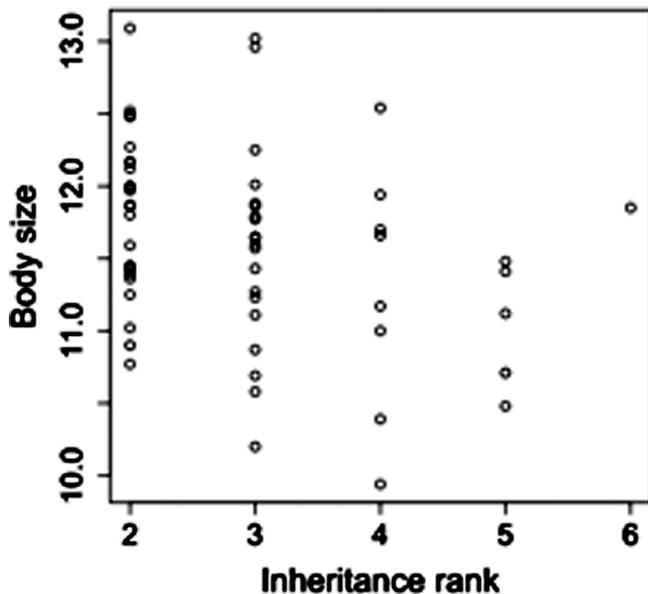


Figure 4

Smaller wasps were ranked significantly lower in the queue to inherit the nest (Rank 2 wasps are first in the queue).

for this pattern are that wasps either use an altogether different cue to discriminate between nest mates or use specific compounds within CHC profiles that correlate closely with relatedness and have not yet been identified. Previous work has identified alkenes and long linear alkanes that alter with social rank in *P. dominulus* (Sledge et al. 2001; Sledge et al. 2004; Dapporto et al. 2007) and it thus seems unlikely that they should also be used to recognize kin. Correspondingly, Dani et al. (2001) identified no role for linear alkanes in kin recognition. Yet, excluding these compounds from our analyses brought no change to our results. A third alternative is that relatives are identified by their CHC profiles in the very early founding period, when wasps emerge from hibernation and social ranks are determined, but quickly become homogenized as wasps build and occupy a nest together (cf. Dapporto et al. 2004). Although we found that CHC profiles do vary with genetic relatedness during the period when we sampled them, it may be that this variation represents only a lingering trace of a more robust

relationship that might guide individual decisions made during the founding period and retained throughout the season.

Our findings are the first to demonstrate that contributions of individual nest mates to the group's foraging effort vary with genetic relatedness to the dominant (and therefore the brood). This effect was dependent on an individual's body size, which we found to be correlated with inheritance rank. Smaller wasps were more likely to be low in the inheritance queue. Our data for relatives of the dominant are in accordance with previous findings that low-ranked wasps forage more (Cant and Field 2001). However, our data suggest an entirely different picture for unrelated subordinates, for whom our model predicted no correlation between foraging effort and body size. Previous empirical work may have failed to detect this effect because interactions between inheritance rank and relatedness were not specifically investigated (Leadbeater et al. 2010) or because variation in relatedness was limited (Cant and Field 2001; Cant et al. 2006a). We are cautious in this interpretation because it assumes that the relationship between size and rank is similar for relatives and nonrelatives, which we have low power to test in this data set. Furthermore, we found that aggression was positively correlated with body size for unrelated subordinates but not for full sister, and this contrasts with previous findings that low-ranked sisters are typically less aggressive (Cant et al. 2006b). Finally, we know the inheritance ranks of only a limited number of wasps within our data set and cannot be sure that the relationship between rank and body size is equivalent for nonrelatives and relatives, but these findings provide a clear avenue for further analysis. In general, they suggest that different subordinates may help for different reasons (Field and Cant 2007). For example, unrelated subordinates may have to "pay to stay" (Kokko and Johnstone 1999, Kokko et al. 2002), whereas the behavior of relatives reflects trade-offs between indirect and direct fitness benefits (Field et al. 2006).

These findings add to parallels between primitively eusocial insect groups and vertebrate cooperative breeders because helping behavior varies with genetic relatedness to the recipient in several vertebrate groups (Russell and Hatchwell 2001; Griffin and West 2003; Komdeur 2003; Cornwallis et al. 2009; Nam et al. 2010; Zottl et al. 2013). In both cooperatively breeding vertebrates and primitively eusocial insects, subordinate helpers face a trade-off between direct and indirect fitness interests (Queller et al. 2000; Cant and Field 2001; Clutton-Brock 2002; West et al. 2002; Leadbeater et al. 2011). This sets them apart from the sterile

workers of advanced eusocial societies, leading to variation in the fitness benefits of cooperative behavior, which can be exploited by individuals that can recognize their own relatedness status.

In summary, our results suggest that the raw material for within-kin recognition based on CHC profiles is available in *P. dominulus*. Nonetheless, we find that genetic relatedness provides a better predictor of social behavior, most probably because the specific components of CHC profiles that wasps use have not yet been determined. Our findings highlight that primitively eusocial cofoundress groups face similar trade-offs in the indirect and direct fitness benefits of social behavior as cooperatively breeding vertebrates, and that these trade-offs lead to greater potential for variation in behavior with relatedness than is typically found in advanced eusocial societies.

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REFERENCES

- Arnold G, Quenet B, Cornuet JM, Masson C, DeSchepper B, Estoup A, Gasqui P. 1996. Kin recognition in honeybees. *Nature*. 379:498–498.
- Arnold G, Quenet B, Masson C. 2000. Influence of social environment on genetically based subfamily signature in the honeybee. *J Chem Ecol*. 26:2321–2333.
- Bates D, Maechler M, Bolker B. 2011. lme4: linear mixed-effects models using Eigen and Eigen. R package version 0.999375-42 [cited 2013 November 29]. Available from: <http://cran.r-project.org/web/packages/lme4/index.html>.
- Bonckaert W, van Zweden JS, d'Ettorre P, Billen J, Wenseleers T. 2011. Colony stage and not facultative policing explains pattern of worker reproduction in the Saxon wasp. *Mol Ecol*. 20:3455–3468.
- Boomsma JJ, d'Ettorre P. 2013. Nice to kin and nasty to non-kin: revisiting Hamilton's early insights on eusociality. *Biol Lett*. 9:20130444.
- Burham KP, Anderson DR. 2002. Model selection and multi-model inference: a practical information-theoretic approach. New York (NY): Springer.
- Cant MA, English S, Reeve HK, Field J. 2006a. Escalated conflict in a social hierarchy. *Proc R Soc B-Biol Sci*. 273:2977–2984.
- Cant MA, Field J. 2001. Helping effort and future fitness in cooperative animal societies. *Proc R Soc B-Biol Sci*. 268:1959–1964.
- Cant MA, Field J. 2005. Helping effort in a dominance hierarchy. *Behav Ecol*. 16:708–715.
- Cant MA, Llop JB, Field J. 2006b. Individual variation in social aggression and the probability of inheritance: theory and a field test. *Am Nat*. 167:837–852.
- Clutton-Brock T. 2002. Breeding together: kin selection and mutualism in co-operatively breeding vertebrates. *Science*. 296:69–72.
- Cornwallis CK, West SA, Griffin AS. 2009. Routes to indirect fitness in cooperatively breeding vertebrates: kin discrimination and limited dispersal. *J Evolution Biol*. 22:2445–2457.
- Crozier RH, Dix MW. 1979. Analysis of two genetic models for the innate components of colony odour in the social hymenoptera. *Behav Ecol Sociobiol*. 4:217–224.
- d'Ettorre P, Lenoir A. 2010. Nestmate recognition. In: Lach L, Parr C, Abbott K, editors. *Ant ecology*. New York (NY): Oxford University Press. p. 194–208.
- Dani FR, Foster KR, Zacchi F, Seppa P, Massolo A, Carelli A, Arevalo E, Queller DC, Strassmann JE, Turillazzi S. 2004. Can cuticular lipids provide sufficient information for within-colony nepotism in wasps? *Proc R Soc London Ser B-Biol Sci*. 271:745–753.
- Dani FR, Jones GR, Destri S, Spencer SH, Turillazzi S. 2001. Deciphering the recognition signature within the cuticular chemical profile of paper wasps. *Anim Behav*. 62:165–171.
- Dapporto L, Bruschi C, Cervo R, Petrocelli I, Turillazzi S. 2010. Hydrocarbon rank signatures correlate with differential oophagy and dominance behaviour in *Polistes dominulus* foundresses. *J Exp Biol*. 213:453–458.
- Dapporto L, Dani FR, Turillazzi S. 2007. Social dominance molds cuticular and egg chemical blends in a paper wasp. *Curr Biol*. 17:R504–R505.
- Dapporto L, Pansolli C, Turillazzi S. 2004. Hibernation clustering and its consequences for associative nest foundation in *Polistes dominulus* (Hymenoptera Vespidae). *Behav Ecol Sociobiol*. 56:315–321.
- Dapporto L, Sledge FW, Turillazzi S. 2005. Dynamics of cuticular chemical profiles of *Polistes dominulus* workers in orphaned nests (Hymenoptera, Vespidae). *J Insect Physiol*. 51:969–973.
- Field J, Cant MA. 2007. Direct fitness, reciprocity and helping: a perspective from primitively eusocial wasps. *Behav Process*. 76:160–162.
- Field J, Cronin A, Bridge C. 2006. Future fitness and helping in social queues. *Nature*. 441:214–217.
- Gadagkar R. 1990. Evolution of eusociality: the advantage of assured fitness returns. *Philos T R Soc B*. 329:17–25.
- Gamboa GJ. 1996. Kin recognition in social wasps. In: Turillazzi S, West-Eberhart MJ, editors. *Natural history and evolution of paper-wasps*. Oxford (UK): Oxford Science. p. 161–177.
- Goslee SC, Urban DL. 2007. The ecodist package for dissimilarity-based analysis of ecological data. *J Stat Softw*. 22:1–19.
- Griffin AS, West SA. 2003. Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. *Science*. 302:634–636.
- Hamilton WD. 1964. The genetical evolution of social behaviour. I&II. *J Theor Biol*. 7:1–52.
- Johnson JB, Omland KS. 2004. Model selection in Ecology and Evolution. *Trends Ecol Evol*. 19:102–108.
- Keller L. 1997. Indiscriminate altruism: unduly nice parents and siblings. *Trends Ecol Evol*. 12:99–103.
- Kokko H, Johnstone RA. 1999. Social queuing in animal societies: a dynamic model of reproductive skew. *Proc R Soc London Ser B-Biol Sci*. 266:571–578.
- Kokko H, Johnstone RA, Wright J. 2002. The evolution of parental and alloparental effort in cooperatively breeding groups: when should helpers pay to stay? *Behav Ecol*. 13:291–300.
- Komdeur J. 2003. Daughters on request—about helpers in the Seychelles warbler. *Proc R Soc London B, Biol Sci*. 270:3–11.
- Konovalov D, Manning C, Henshaw MT. 2004. KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. *Mol Ecol Notes*. 4:779–782.
- 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.
- Leadbeater E, Carruthers JM, Green JP, van Heusden J, Field J. 2010. Unrelated helpers in a primitively eusocial wasp: is helping tailored towards direct fitness? *Plos One*. 5:e11997.
- Nam K-B, Simeoni M, Sharp SP, Hatchwell BJ. 2010. Kinship affects investment by helpers in a cooperatively breeding bird. *Proc R Soc London, Ser B*. 277:3299–3306.
- Nehring V, Evison SEF, Santorelli LA, d'Ettorre P, Hughes WOH. 2011. Kin-informative recognition cues in ants. *Proc R Soc B-Biol Sci*. 278:1942–1948.
- Page RE, Metcalf RA, Metcalf RL, Erickson EH, Lampman RL. 1991. Extractable hydrocarbons and kin recognition in honeybee (*Apis mellifera* L.). *J Chem Ecol*. 17:745–756.
- Pardi L. 1942. Ricerche sui Polistinae V. La poliginia iniziale di *Polistes gallicus* L. *Boll Ist Entomol Univ Bologna*. 14:1–106.
- Queller DC, Zacchi F, Cervo R, Turillazzi S, Henshaw MT, Santorelli LA, Strassmann JE. 2000. Unrelated helpers in a social insect. *Nature*. 405:784–787.
- Ratnieks FLW, Helanterä H, Foster KR. 2007. Are mistakes inevitable? Sex allocation specialization by workers can reduce the genetic information needed to assess queen mating frequency. *J Theor Biol*. 244:470–477.
- Russell AF, Hatchwell BJ. 2001. Experimental evidence for kin-biased helping in a cooperatively breeding vertebrate. *Proc R Soc, Ser B*. 268:2169–2174.

- Shreeves G, Cant MA, Bolton A, Field J. 2003. Insurance-based advantages for subordinate co-foundresses in a temperate paper wasp. *Proc R Soc B-Biol Sci.* 270:1617–1622.
- Sledge MF, Boscaro F, Turillazzi S. 2001. Cuticular hydrocarbons and reproductive status in the social wasp *Polistes dominulus*. *Behav Ecol Sociobiol.* 49:401–409.
- Sledge MF, Trinca I, Massolo A, Boscaro F, Turillazzi S. 2004. Variation in cuticular hydrocarbon signatures, hormonal correlates and establishment of reproductive dominance in a polistine wasp. *J Insect Physiol.* 50:73–83.
- Turillazzi S, Sledge MF, Moneti G. 1998. Use of a simple method for sampling cuticular hydrocarbons from live social wasps. *Ethol Ecol Evol.* 10:293–297.
- van Zweden JS, Brask JF, Christensen JH, Boomsma JJ, Linksvayer TA, d'Ettore P. 2010. Blending of heritable recognition cues among ant nestmates creates distinct colony gestalt odors but prevents within-colony nepotism. *J Evol Biol.* 23:1498–1508.
- van Zweden JS, Viikainen E, d'Ettore P, Sundstrom L. 2011. Do cuticular hydrocarbons provide sufficient information for optimal sex allocation in the Ant *Formica exsecta*? *J Chem Ecol.* 37:1365–1373.
- West SA, Pen I, Griffin AS. 2002. Cooperation and competition between relatives. *Science.* 296:72–75.
- Zanette L, Field J. 2009. Cues, concessions, and inheritance: dominance hierarchies in the paper wasp *Polistes dominulus*. *Behav Ecol.* 20:773–780.
- Zanette LRS, Field J. 2008. Genetic relatedness in early associations of *Polistes dominulus*: from related to unrelated helpers. *Mol Ecol.* 17:2590–2597.
- Zöttl M, Heg D, Chervet N, Taborsky M. 2013. Kinship reduces alloparental care in cooperative cichlids where helpers pay-to-stay. *Nature Commun.* 4:1341.