Preferential phenotypic association linked with cooperation in paper wasps

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Abstract

Animals can influence their social environment by preferentially associating with certain conspecifics. Such preferential association has gained increasing theoretical attention, as it may influence social evolution and population dynamics. However, relatively little empirical work has examined the occurrence of preferential association and its effects on cooperative group formation. Here, we test the factors associated with cooperative group formation in *Polistes dominulus* nest-founding queen wasps. *P. dominulus* are a good system to study preferential association, as foundresses can nest alone or in groups and group membership is flexible. We found that both social and environmental factors were associated with partner choice. First, facial patterns were associated with cooperation. Wasps with more similar facial patterns were more likely to cooperate than wasps with less similar facial patterns. This preferential phenotypic association fits the theoretical criteria for the evolution of tag-based cooperation. Season was also associated with cooperation; wasps on early-season nests were more likely to cooperate than wasps on late-season nests. High levels of aggression by nest owners during initial interactions were also correlated with lower probabilities of subsequent cooperation, suggesting that nest owners have some control over group membership. Other factors including body weight, weight similarity and nest productivity were not linked with cooperation. Overall, multiple factors influence cooperation in paper wasps, including facial pattern similarity. The occurrence of preferential phenotypic association in paper wasps is quite interesting and may influence the evolution of cooperation and population divergence in this group.

Introduction

The selective environment an individual experiences is strongly influenced by interactions with conspecifics (West-Eberhard, 1983; Wolf *et al.*, 1999). For example, the genotype and phenotype of cooperative group members and potential rivals influence an individual's fitness (McGlothlin *et al.*, 2010); an individual that dominates one group of conspecifics may be subordinate in another group of conspecifics. Consequently, there is growing interest in the factors that influence an individual's social environment, particularly how individuals can change their own social environment

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by preferentially associating with some individuals and avoiding others. Models suggest that preferential association can have important ramifications. In particular, whether or not individuals preferentially associate with conspecifics that have similar phenotypes influences two aspects of evolution: population divergence (Hochberg *et al.*, 2003) and cooperative evolution (Riolo *et al.*, 2001; Antal *et al.*, 2009; Gardner & West, 2010).

Much previous work on population divergence has focused on assortative mating, but recent work suggests that assortative phenotypic association in social contexts may produce similar effects on population structure (Schluter, 2000). Specifically, preferential association between conspecifics with similar phenotypes (preferential phenotypic association, PPA) can lead to viscous populations where phenotypically similar individuals separate into spatially distinct groups,

thereby producing population divergence (Hochberg *et al.*, 2003). There is some empirical evidence of preferential phenotypic association in social species. Male side-blotched lizards with blue phenotypes preferentially settle near other blue males and exhibit some altruistic behaviour towards blue conspecifics (Sinervo *et al.*, 2006). Further, recent comparative analyses suggest that phenotypic polymorphisms are associated with speciation (Hugall & Stuart-Fox, 2012). Although many potential mechanisms could produce speciation in polymorphic species, preferential association between similar morphs is one intriguing possibility. Therefore, preferential phenotypic association among nonmating social partners may be important for understanding population dynamics (Sinervo *et al.*, 2006; Corl *et al.*, 2010). However, there are currently very few empirical examples of preferential phenotypic association of unrelated individuals, so it is difficult to assess how widespread this mechanism may be.

One context where preferential phenotypic association has been studied extensively is kin recognition, although different terms such as 'phenotype matching' are often used in the kin recognition literature (Lacy & Sherman, 1983). In many species, individuals use phenotypic similarity to discriminate between kin and nonkin. Individuals learn their own phenotypes or the phenotypes of known relatives. Later, they treat individuals with similar phenotypes as kin. For example, spadefoot toad tadpoles use similarity in chemical cues to identify kin and are less likely to cannibalize kin than nonkin tadpoles (Pfennig *et al.*, 1993). Likewise, many social insects use similarity in chemical cues to identify kin (Nonacs, 2011).

Preferential phenotypic association may also facilitate the evolution of cooperation. Tag-based cooperation models indicate that stable cooperation can evolve if individuals preferentially associate with those which have similar 'tags' (phenotypes; Riolo *et al.*, 2001). Cooperation based on phenotypic similarity is relatively easy to understand when phenotypic similarly reflects pedigree relatedness. However, models suggest that phenotypic similarity can also promote cooperation when the tags do not reflect overall relatedness. Theoretical work

on tag-based cooperation and its relationship with kin selection has been prolific and controversial (Axelrod *et al.*, 2004; Jansen & van Baalen, 2006; Gardner & West, 2007; Antal *et al.*, 2009). However, relatively little empirical work has tested whether species preferentially cooperate with conspecifics that have similar phenotypes, regardless of pedigree relatedness. There are a few, striking examples where chemical tags influence association (e.g. social amoeba and red fire ants; Keller & Ross, 1998; Queller *et al.*, 2003). However, additional work across taxa and signalling modalities is important to establish the generality of preferential phenotypic association.

Polistes dominulus paper wasps are a good model for studying the occurrence of association based on phenotypic characteristics, as there is extensive variation in cooperative behaviour (Roseler, 1991) and nest-founding queens have obvious phenotypic tags throughout their range (Tibbetts, 2004; Tibbetts *et al.*, 2011b). Some nest-founding wasp queens nest in cooperative groups, whereas others nest alone. Foundresses typically interact with many potential cooperative partners in the first few weeks of the nesting cycle before forming stable cooperative groups (Nonacs & Reeve, 1995; Zanette & Field, 2011). Some cooperative foundresses are related, but many cofoundresses are unrelated (Queller *et al.*, 2000; Zanette & Field, 2008). Foundress cooperation provides substantial benefits to both dominants and subordinates. Cooperation increases nest and individual survival as well as offspring production (Tibbetts & Reeve, 2003; Sumner *et al.*, 2010). Low-ranking individuals within cooperative groups have relatively little direct reproduction (Reeve, 1991; Roseler, 1991), although low-ranking individuals receive inclusive fitness benefits when they are related to dominants (Leadbeater *et al.*, 2011). Further, both related and unrelated subordinates may receive direct reproduction if the dominant foundress dies (Queller *et al.*, 2000).

Polistes dominulus paper wasps have obvious phenotypic tags, variable black facial patterns that function as a signal of agonistic ability. Wasps with more broken black facial patterns are more likely to win fights than individuals with less broken black facial patterns (Fig. 1; Tibbetts & Dale, 2004; Tibbetts *et al.*, 2011a).

Fig. 1 Portraits of four *Polistes dominulus* paper wasps, arrayed from low to high facial pattern brokenness.

Further, facial patterns are used to assess rivals prior to engaging in social interactions (Tibbetts & Lindsay, 2008; Tibbetts *et al.*, 2010; but see Green & Field, 2011). Individuals with similar facial patterns may be more related than expected by random chance, as facial patterns are more similar within offspring from the same nest than across offspring from different nests (Tibbetts, 2006, 2010). Although much of this similarity is due to similarity in rearing environment within a nest, facial pattern is also influenced by genotype (Tibbetts, 2010).

This study will test whether *P. dominulus* foundresses preferentially associate with individuals that have similar facial patterns. First, we test whether foundresses in wild associations have more similar facial patterns than would be expected by random chance. Second, we experimentally test the factors that influence association by pairing unrelated foundresses and assessing whether or not they choose to associate. We also test whether other factors are associated with cooperative group formation, including body weight, weight similarity, time of season, nest size and aggression during initial interactions.

Materials and methods

Wild nests

Wild multiple foundress nests were collected from 16 different sites around Ann Arbor, MI, in May 2011. A total of 57 nests were collected, and the number of foundresses per nest ranged from two to eight. After collection, foundresses were weighed on a scale accurate to 0.001 g and their facial patterns were photographed for facial pattern analysis.

Experimental association

For the association experiment, single foundresses were collected from sites around Ann Arbor, MI. Collections were performed both early and late in the season. Early collections were performed during the first week of May, approximately 1 week after nest foundation. Late collections were performed in June, approximately 3 weeks before worker emergence. After collection, wasps were processed by weighing on a scale accurate to 0.001 g and facial patterns were photographed. Wasps were also marked with silver or orange enamel paint on the top of their thorax for individual identification. In addition, the size of each nest was measured by counting the number of nest cells. Each foundress was used in a single trial, and there was no reuse of foundresses or nests across trials.

Within a week of collection, two foundresses from sites at least 5 km apart were placed together in a single box. *Polistes* are usually philopatric, although they have been observed to disperse up to 300 m (Makino *et al.*,

1987). As a result, using wasps collected from distant locations ensures they had not previously interacted. In each pair, a randomly selected wasp was chosen as the owner, and the owner's nest was glued to the top of the container. The other wasp was the potential joiner. This experimental set-up mimics wild conditions, as *P. dominulus* foundresses frequently move between nests before worker emergence (Reeve, 1991; Nonacs & Reeve, 1995). One study found that approximately 75% of all foundresses switch nests during the first 12 days of the founding period and visit approximately three nests before settling down to cooperate (Pratte, 1979).

The first hour of social interactions between the foundresses was videotaped. Later, the videotapes were scored for number of aggressive acts, including bites, mounts and grapples. Both foundresses remained in the same box for 10 days. During this period, the behaviour of each foundress was scored four times a day. An observer blind to experimental predictions scored whether each wasp was on the nest with their conspecific, on the nest alone or off the nest. Association time was calculated as number of observations where wasps were together on the nest/total observations. Trials were then categorized as 'cooperative' ($n = 47$) or 'non-cooperative' ($n = 98$) based on the average time spent in association, with 5% time in association used as the cut-off. The cut-off could be moved anywhere between 3% and 30% time in association without changing the results, and 146 association trials were performed. One foundress died during the trials, so the final sample size was 145.

Facial pattern analysis

We assessed the facial pattern of each individual by analysing a digital picture of the wasp's face with Adobe Photoshop. A wasp's facial pattern 'brokenness' reflects the amount of disruption of the black coloration in the central part of the face (Fig. 1). Previous work has shown that brokenness is a condition-dependent signal of agonistic ability that is used to minimize the costs of conflict during contests (Tibbetts, 2010; Tibbetts *et al.*, 2010, 2011a). First, the area of the clypeus containing the population-wide badge variability was converted into a 30 × 60 pixel bitmap. Then, the number of pixels containing black pigment within each vertical column along the horizontal length of the clypeus was counted. We were interested in the total disruption of the black facial pattern, so we calculated the standard deviation of the black pigment deposition from pixels 5 to 55 along the horizontal gradient of the clypeus. We excluded the first and last five pixels from the brokenness analysis because the edges of the clypeus are black. As a result, wasps with black spots in the first and last five pixels have facial patterns that appear less broken than individuals with black spots that extend to

the edge of the clypeus. The standard deviation of the black pigment deposition, or 'brokenness' of a wasp's face, measures the amount of disruption in the black coloration and a signal of fighting ability. Facial pattern brokenness is also referred to as 'advertised quality', with high brokenness reflecting high advertised quality.

Statistical analysis

Data were analysed using SPSS v. 19 (IBM, Armonk NY, USA). Similarity in wild foundress associations was measured using likelihood ratio test. Collection location was originally included as a random effect in the model, but it accounted for a negligible amount of variation, so was removed from the final model.

The factors that influenced association in the laboratory experiment were tested using a generalized linear model with a binary outcome. Cooperative vs. noncooperative was the dependent variable. Independent variables were nest size (number of cells), time of season (early vs. late), foundress facial pattern brokenness, foundress facial pattern similarity (absolute value of owner–joiner facial pattern brokenness), foundress weight, foundress weight similarity (absolute value of owner–joiner weight), the number of aggressive acts initiated by the nest owner and the number of aggressive acts initiated by the nest joiner. Aggressive acts were measured as the sum of mounts, bites and grapples in the first hour of interactions and were log-transformed. The following two-way interactions were also tested: the interaction between owner and joiner facial pattern brokenness, the interaction between owner and joiner weight and the interaction between season and nest size. None of the two-way interactions were significant, so they were removed from the final model using backward stepwise elimination, however, they are reported below.

Results

Wild nests

In wild nests, facial pattern was more similar within a cofoundress association than across the population (Fig. 2, $\chi^2 = 6.95$, $P < 0.001$). Thirty-two percent of the total variation in face within the sample is attributable to nest. However, weight was not more similar within cofoundress associations than across the population ($\chi^2 = 1.59$, $P = 0.21$).

Association experiment

Whether or not foundresses formed cooperative associations was influenced by both environmental and social factors (Table 1). Cooperation was strongly associated with season, with early-season associations producing more cooperation than late-season associations. Foun-
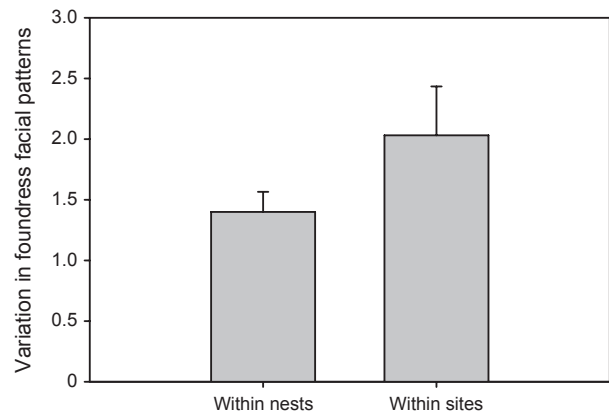

Fig. 2 Mean (± SE) variation in foundress facial patterns within a foundress association vs. within all multiple foundress nests at a single location. Variation is measured as standard deviation.

Table 1 Generalized linear model of factors significantly associated with cooperation in *Polistes dominulus*.

Fixed effect	B	Wald χ^2	P-value
Time of season (early–late)	−1.4	7.78	0.005
Facial pattern similarity	0.23	4.72	0.03
Owner to joiner aggression	1.57	3.98	0.046

dress characteristics also influenced cooperative behaviour. Foundresses with similar facial patterns spent more time associating than foundresses with different facial patterns (Fig. 3). The first hour of interactions between foundresses was negatively linked with cooperative behaviour over the next 10 days. In particular, nest owners that directed less aggression towards joining foundresses were more likely to form cooperative associations with the joiners than nest owners that directed more aggression towards joining foundresses (Fig. 4b). However, aggression initiated by joining foundresses was not associated with subsequent cooperation (Fig. 4a). This suggests that the nest owner has some control over joining behaviour. The following factors were not linked with cooperative behaviour (Table 2): nest size, cofoundress facial patterns, cofoundress weight, similarity in foundress weight, the interaction between cofoundress facial patterns, the interaction between cofoundress weights and the interaction between time of season and nest size.

Discussions

Facial pattern similarity and association

In the wild, there is greater facial pattern similarity within foundresses from a nest than between foundresses from different nests (Fig. 2). The similarity could be a by-product of relatedness among cofoundresses (Queller
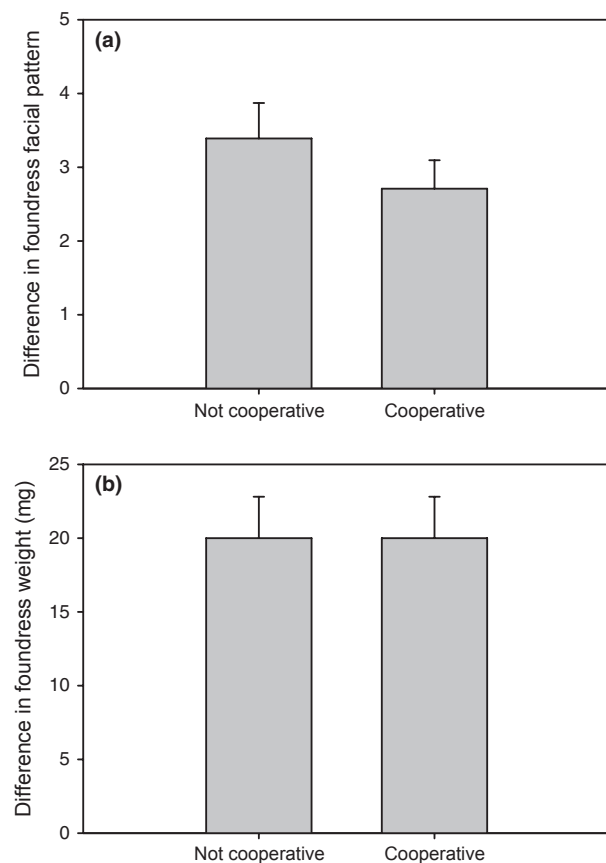
Fig. 3 Difference (mean ± SE) in the (a) facial pattern brokenness and (b) weight (in mg) of foundress pairs that did not cooperate vs. formed cooperative associations. Difference calculated as absolute value of nest owner minus nest joiner.

Fig. 4 Mean (± SE) aggression during the first hour of interactions (a) from nest joiner to owner and (b) from nest owner to joiner in foundress pairs that did not cooperate vs. formed cooperative associations. Aggression includes bites, mounts and grapples.

et al., 2000; Zanette & Field, 2008), as offspring facial patterns are more similar within than between nests (Tibbetts, 2006, 2010). Alternatively, similarity may be intentional rather than merely a by-product, as foundresses may choose to preferentially associate with individuals that have similar facial patterns, regardless of relatedness.

The results of the association experiment suggest that at least some of the similarity in cofoundress facial patterns is likely due to PPA. Wasps with similar facial pattern brokenness were more likely to associate than wasps with different facial pattern brokenness (Fig. 3), although all foundresses in the experiment were thought to be unrelated and had no prior history of interactions. The occurrence of PPA in paper wasps is quite interesting, as only a handful of other studies have identified PPA among unrelated individuals in 'advanced' social species. This preferential phenotypic association may have important consequences for the evolutionary stability of cooperation as well as population dynamics.

Why do paper wasps preferentially associate with conspecifics that have similar facial patterns? PPA could
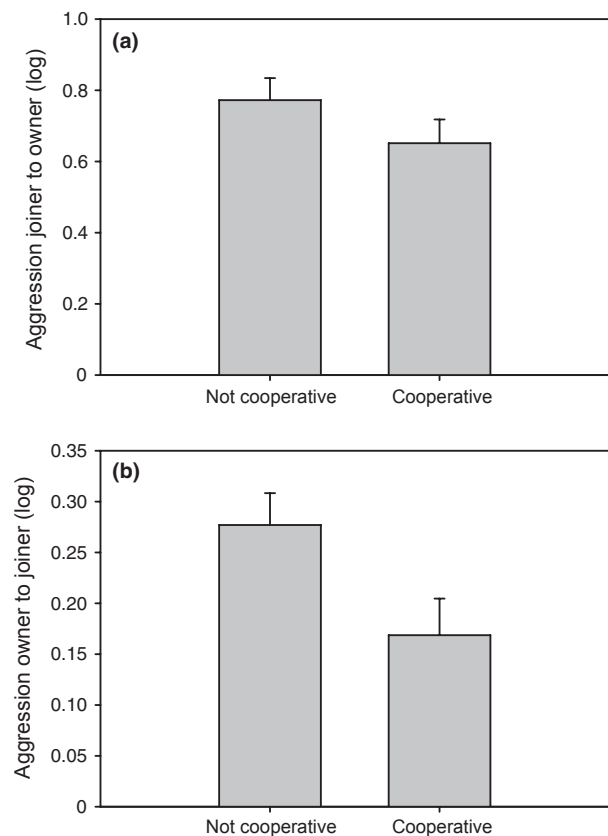
Table 2 Generalized linear model of factors not significantly associated with cooperation in *Polistes dominulus*. Nonsignificant interaction terms are removed from the final model through backwards stepwise elimination; however, they are reported below.

Fixed effect	Wald χ^2	P-value
Number of nest cells	0.10	0.75
Owner facial pattern	0.15	0.70
Joiner facial pattern	1.09	0.29
Owner weight	1.44	0.23
Joiner weight	2.95	0.09
Weight similarity	2.76	0.10
Joiner to owner aggression	0.48	0.49
Time of season × nest size	2.1	0.15
Owner × joiner weight	0.50	0.48
Owner × joiner facial pattern	2.63	0.10

arise if facial patterns provide information about pedigree relatedness, as choosing related social partners provides substantial inclusive fitness benefits (Hamilton, 1964). Paper wasps that eclose from the same nest have

more similar facial patterns than expected by random chance (Tibbetts, 2006, 2010). Therefore, choosing to associate with conspecifics that share similar facial patterns may increase the likelihood of choosing related social partners in the wild. At the same time, there is extensive diversity in facial patterns within nests, as well as variation in relatedness within cofoundress associations (Queller *et al.*, 2000; Zanette & Field, 2008). As a result, future work examining how much information facial patterns provide about relatedness in wild foundress associations will be important. For example, do related cofoundresses have more similar facial patterns than unrelated foundresses? Do unrelated cofoundresses share greater facial pattern similarity than expected by random chance? Importantly, high pedigree relatedness associated with phenotypic tags is not required for PPA to evolve. Models suggest that PPA can originate due to cooperative benefits, whether or not phenotypes provide information about relatedness (Antal *et al.*, 2009).

Wasps could also use chemical signals to identify related cofoundresses (Gamboa, 2004), although chemical signals may not provide useful information about relatedness during the period of nest foundation (Dapporto *et al.*, 2004). Chemical signals change rapidly with the environment (Howard & Blomquist, 2005), so individuals that overwinter in the same hibernacula have similar chemical profiles, regardless of true relatedness (Dapporto *et al.*, 2004). Facial patterns are static (E.A. Tibbetts, unpublished data), so they may provide more reliable information about relatedness than cuticular hydrocarbons.

Effective PPA requires that individuals 'know' their own phenotype and can compare it with the facial pattern of conspecifics. Previous work in agonistic contexts in *P. dominulus* illustrates that wasps behave as if they 'know' their own facial patterns. In addition, wasps compare their own facial patterns to that of conspecifics when making decisions during aggressive competition (Tibbetts *et al.*, 2010). Currently, the mechanism used for assessing one's own facial pattern is not clear. There is some facial pattern similarity within wasps on a nest, so wasps could estimate their own facial patterns by remembering the phenotypes of individuals from their natal nest (Lacy & Sherman, 1983). Alternatively, wasps may use physiological correlates, such as hormone titres, to estimate their facial patterns (Tibbetts *et al.*, 2013). Juvenile hormone titres are correlated with facial pattern brokenness (Tibbetts *et al.*, 2011a), and juvenile hormone titres also directly influence contest behaviour (Tibbetts & Izzo, 2009) and self-assessment prior to engaging in social competition (Tibbetts *et al.*, 2013). This and other research show that paper wasps have the behavioural capacity to perform PPA based on facial patterns, although additional work will be useful to identify the mechanism by which wasps identify their facial pattern.

Preferential phenotypic association provides a mechanism that may stabilize cooperation. Models indicate that evolutionarily stable cooperation requires preferential association among altruists (Hamilton, 1964; Kerr & Godfrey-Smith, 2002). One way for this to occur is through preferential cooperation between conspecifics that have similar phenotypes. The role of PPA in cooperation has been addressed from multiple perspectives, using different terms for PPA (the green-beard effect, the armpit effect (or genetic kin recognition), tag-based cooperation and cooperation based on phenotypic similarity). These models make different assumptions about the relationship between phenotypic and genetic similarity and come to different conclusions about whether pedigree relatedness or population structure is required for PPA to mediate stable cooperation (Riolo *et al.*, 2001; Axelrod *et al.*, 2004; Antal *et al.*, 2009; Gardner & West, 2010; Nonacs, 2011). Thus far, empirical work on PPA and cooperation has lagged behind theoretical development.

The occurrence of PPA in paper wasps suggests that it may play a role in cooperation on paper wasp nests. However, a major challenge of empirical work on PPA and cooperation is that it is difficult to test whether PPA influences the evolution of cooperation within a particular species. Models typically examine how PPA favours the evolution of cooperation generally rather than creating critical predictions that can be tested in a single species. Comparative analyses provide a useful framework for exploring whether PPA influences cooperative evolution. Specifically, if PPA via variable facial patterns facilitates the evolution of cofoundress cooperation, cooperation among foundresses should be more common in species with variable facial patterns. This prediction is supported, as variable facial patterns are confined to species with cooperative foundress associations (Tibbetts, 2004). However, there are alternative explanations for the relationship between variable facial patterns and cooperation. Facial pattern variation that may evolve after cooperation appears to promote nepotism or to increase the sophistication of cooperation. Alternatively, facial patterns may be a green-beard trait that spreads via runaway social selection in cooperative species (Nonacs, 2011). Of course, facial patterns function in social signalling contexts other than PPA (Tibbetts, 2002; Tibbetts & Lindsay, 2008; Tannure-Nascimento *et al.*, 2008; Tibbetts & Sheehan, 2011), so it is difficult to establish which factors are the driving force in the coevolution of tags and cooperative behaviour. Future work testing whether PPA based on facial patterns occurs in other paper wasps will be useful to test whether PPA may commonly be involved in the evolution of cooperation in paper wasps.

Preferential phenotypic association may also contribute to population divergence and speciation. Theory suggests that PPA may produce viscous populations where phenotypically similar individuals separate into

spatially distinct groups (Hochberg *et al.*, 2003). Although models suggest that PPA may produce these effects in contexts similar to that identified in this study, empirical evidence for the relationship between PPA and population divergence is sparse. There is some evidence for its role in species with discrete polymorphisms (Sinervo *et al.*, 2006; Corl *et al.*, 2010). However, it is currently unclear how frequently PPA will produce viscous populations, assortative mating and reduced gene flow. In *P. dominulus*, the large geographic range is characterized by substantial differences in facial patterns. Although facial pattern variation occurs across the entire range of *P. dominulus*, there is geographic variation in the type and extent of variation (Tibbetts *et al.*, 2011b). The combination of striking phenotypic divergence across their range and PPA suggests that PPA could be involved in paper wasp divergence. However, phenotypic divergence in paper wasps may be influenced by environmental differences and/or genetic divergence (Tibbetts *et al.*, 2011b; Green *et al.*, 2012). Previous work has not explicitly tested the extent of genetic divergence across the geographic range of *P. dominulu*s. Future work testing the relationship between phenotypic and genetic divergence will be a useful step. Although a correlation between phenotypic and genotypic divergence does not critically test whether PPA is involved in population divergence, it supports the hypothesis that PPA in paper wasps could play a role in population structure. In addition, comparative analyses will be important to assess whether there is accelerated speciation or divergence in species with variable facial patterns.

One interesting aspect of studying PPA in paper wasps is that the phenotype is functional rather than neutral. Models that examine the consequences of PPA typically do not consider whether the phenotypes have alternative functions in other contexts, although other functions could clearly influence the evolutionary dynamics of PPA. Neutrality of the phenotype is not required, as previous empirical work on PPA in the context of speciation has examined polymorphisms that are functional in other contexts (Sinervo *et al.*, 2006; Corl *et al.*, 2010). For example, the blue morph in side-blotched lizards functions as a sexually selected signal, and variation in throat colour is associated with behavioural and hormonal differences (Sinervo & Calsbeek, 2006).

Other factors linked with association behaviour

The formation of cooperative associations was also influenced by season. Foundresses on early-spring nests were more likely to form cooperative associations than foundresses on late-season nests. This result matches previous studies on wild nests, as nest membership is flexible at the beginning of the season and becomes relatively stable as the colony cycle progresses (Nonacs &

Reeve, 1995). Decreased willingness to accept potential joiners as the season progresses could be the behavioural mechanism that mediates the change in stability of nest membership. Functionally, early-season joiners are likely to provide a greater reproductive benefit than late joiners. Joiners increase nest and individual survival during the founding stage, but are thought to provide little benefit after worker emergence (Reeve, 1991; Tibbetts & Reeve, 2003). In fact, cofoundresses often disappear after worker emergence, and there is some evidence that the disappearance may be a result of eviction (Gamboa *et al.*, 1999 but see Field & Cant, 2009). Therefore, the relationship between season and association behaviour matches theoretical predictions as well as previous observational studies in the wild.

Interestingly, nest size was not linked with cooperation behaviour. Models of reproductive sharing in groups often predict that the formation of stable cooperative groups will depend, in part, on group productivity (Reeve & Ratnieks, 1993; Reeve *et al.*, 2000). Paper wasp nest size is a good proxy for productivity, as each nest cell produces one offspring. Nevertheless, nest size was not significantly linked with association behaviour, suggesting that other factors have stronger effects on cooperation than nest size. Nest size is linked with association if season is removed from the statistical model, likely because early-season nests are smaller than late-season nests.

There was also a negative relationship between nest owner aggression and association behaviour (Fig. 4), suggesting that nest owners have some control over whether or not other individuals join their nest. Nest owners that are more aggressive towards potential joiners are less likely to be part of stable cooperative associations than nest owners that are less aggressive. In contrast, aggression initiated by potential joiners was not associated with the formation of cooperative groups. In many social groups, there are questions about whether insiders or outsiders control group membership (Johnstone & Cant, 1999; Reeve & Emlen, 2000). Previous work in paper wasps typically assesses group formation via regular censuses to test how wasps move between nests (Nonacs & Reeve, 1995; Zanette & Field, 2011; Seppa *et al.*, 2012). As a result, relatively little is known about behavioural dynamics during group formation (Tibbetts & Shorter, 2009). However, work in other species shows that eviction of potential group joiners is quite common (e.g. Buston, 2003). This result also matches previous work in other species, suggesting that ownership often influences contest outcomes (Davies, 1978).

Overall, multiple social and environmental factors influence the formation of cooperative groups in paper wasps. Most interestingly, wasps preferentially cooperate with conspecifics that have similar facial patterns. Preferential association with conspecifics that have similar phenotypes has important theoretical ramifications,

but little previous empirical work has tested for its occurrence. Signals are often studied in a single context. For example, *P. dominulus* facial patterns are typically considered in the context of aggressive signalling (Tibbetts & Dale, 2004; Tibbetts & Izzo, 2010). However, considering how species use these signals across multiple contexts is an important area of future research that may have implications for understanding diverse topics including population dynamics, speciation and cooperative evolution.

Acknowledgments

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