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Sociality outside the nest: helpers in pre-hibernating clusters of *Polistes dominulus*

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After mating and leaving the maternal nest, gynes of several paper wasp species living in temperate climates aggregate in sheltered places. Some authors state that the social stage of paper wasps ends at the beginning of autumn. Here, we show that the death of workers and the abandonment of the nest do not imply the end of the social phase in *Polistes dominulus*, and that many social interactions also occur in pre-hibernating clusters. In particular, a few individuals performed external tasks, i.e. foraging and providing food via trophallaxis to other wasps. These "helpers" died early in winter, as workers generally do, but they were fertilised like gynes.

Introduction

Group living does not necessarily involve social interactions (such as dominance relations, division of labour, food exchange). However, in many insect species, individuals group together for various reasons. The benefits of group living are related to mating, coping with harsh environmental conditions (winter survival) and defence (dilution effect) (Eibl-Eibesfeldt 1967, Masters *et al.* 1988, Sillen-Tullberg 1988, Wells *et al.* 1990). These aggregations range from just a few to hundreds or thousands of individuals.

Gynes of *Polistes* wasps living in temperate zones emerge from the nest in late summer, mate and then hibernate during the cold season (Reeve 1991). Females of several species usually hibernate in clusters (Rau 1931, Pardi 1942, Yoshikawa 1963). This phase of the life cycle is divided into three stages: pre-hibernating, hibernating and post-hibernating (reviewed in Yoshikawa 1963). In the pre-hibernating stage, the wasps aggregate in the corners of eaves, ceilings and beams (Pardi 1942, Yoshikawa 1963). In Mediterranean areas, wasps generally pre-hibernate close to conspicuous food resources such as vines and figs (L. Dapporto pers. obs. for *P. dominulus*). In Mediterranean populations of *P. dominulus*, the pre-hibernating stage can be observed from September to early November (Pardi 1942). The shelters used during this phase are not the hibernacula (Rau 1931, Pardi 1942, Yoshikawa 1963). Usually, *Polistes* wasps spend the hibernating period (the second stage) in safer quarters such as rock crevices, curved tiles and hollow bricks (Rau 1931, Pardi 1942). As the temperature rises in early spring, the wasps become active and the post-hibernating stage begins. This final stage ends with nest construction (Yoshikawa 1963).

Overwintering is a critical period for females; in fact, many of them die before spring (Rau 1930, Pratte 1982, Starks 2001, Gamboa *et al.* 2004). Nourishment and resource storage seem to play a fundamental role in winter survival (Strassmann 1979, Hunt *et al.* 2003). Indeed, in the pre-hibernating stage, wasps are still active and forage for nectar and fruits. Yet trophallaxis and other behaviours occurring at the nest have never been reported (Gonzalez *et al.* 2002). According to Yoshikawa (1963), the social stage of the paper wasp colony cycle ends with the death of workers, due to the lack of food exchange between reproductive individuals during autumn. However, in most *Polistes* species, it is not always possible to distinguish workers from foundresses (Reeve 1991, O'Donnell 1998). Furthermore, "workers" do not necessarily die during winter; in fact, it has been demonstrated in *P. fuscatus* (Reeve *et al.* 1998) and in *P. dominulus* (Starks 2001) that some of the first emerging "workers" leave their natal nests, hibernate, and found nests in spring.

Interestingly, in *P. dominulus* we see that not all workers behave as workers (Starks 2001) and not all foundresses (or queens) behave as foundresses (Queller *et al.* 2000). Instead of reproducing, some spring females join conspecifics in cooperative associations where subordinates may not reproduce (Queller *et al.* 2000), thereby behaving more similar to workers than to queens. According to the sub-fertility hypothesis (West-Eberhard 1975), some individuals might emerge with a reduced reproductive capacity and become helpers. Yet, there has been little support for this hypothesis (Reeve 1991). Röseler *et al.* (1984) showed, however, that some predisposition to become dominant might be present even on the very first day after hibernation in *P. dominulus*. These findings led Reeve (1991) to suggest that some interactions between gynes may already occur in autumn aggregations. Here, we investigate if the abandonment of nests really implies the end of the social phase, or if some interactions and division of labour also occur in pre-hibernating clusters.

Material and methods

In September 2003, we collected three clusters (C: $n = 48$, R1: $n = 82$, R2: $n = 87$) of *Polistes dominulus* at the beginning of the pre-hibernating stage from sites at Cavriglia and Reggello (Tuscany, Italy). The wasps were aggregated in open quarters exposed to sunlight (the external part of a chimney, an old open pot). We individually marked the wasps on the wings with different colour combinations. We caged them in containers (50 × 50 × 50 cm) consisting of a large wood frame closed by three glass and two net sides. The net sides prevented overheating in the cages. The corners of the large frame offered appropriate shelters for the wasps. The containers were placed in an open gallery and the animals received direct sunlight in the early afternoon. This setting is quite similar in terms of temperature, humidity and light exposure to those from which the aggregations were collected. The wasps were supplied with water and sugar.

We selected nine behavioural items: dominance performed and received, aggression performed and received, food request performed and received, trophallaxis obtained and given, and foraging on the sugar. In dominance behaviour, the dominant wasp climbs on and intensively antennates the body of the subordinate wasp, which remains motionless. In food request, an individual climbs on the body of another and asks for food by mouth-to-mouth contact. Trophallaxis occurs when a wasp gives liquid food to another via mouth-to-mouth contact. Attacks include lunges, bites, aggressive mounts and chases. We recorded dominance, food request, aggression and trophallaxis behaviours by the "all occurrences sampling method" (Altmann 1974). During each "all occurrences" session (lasting 20 min), we recorded the behaviour, actor and receiver. We used the "scan animal sampling method" (Altmann 1974) at 5-min intervals to record the individuals foraging on the sugar. Due to the shortness of the pre-hibernating period (usually no more than 40 days) and the restriction of daily activity of the wasps (a few hours on warm sunny days), we were only able to perform a total of 20 hours of observations. Until 15 December 2003 (just before the rigours of winter), we checked the R1 and R2 cages daily for dead wasps, which were dissected to detect the presence of sperm in the spermatheca.

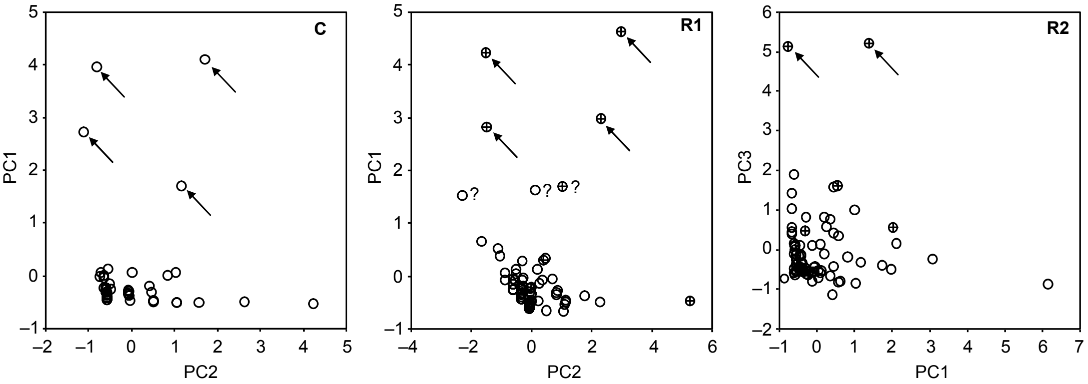

Fig. 1. Principal Component Analyses in the three aggregations (C, R1, R2). PC1 for C and R1, PC3 for R2 represent trophallaxis given and foraging activity (helping behaviour). Helpers (indicated by arrows) are the individuals showing higher values in *y*-axes. The individuals showing intermediate characteristics are indicated with a question mark (?). Individuals marked by a cross died before the beginning of winter.

Principal Component Analysis (PCA) was applied to the data set of the nine behavioural variables for each aggregation. PCA allows the separation of individuals on the basis of a set of correlated behaviours. Kaiser-Meyer-Olkin (KMO) and Bartlett's tests were used to assess sampling adequacy. We removed the variables presenting at least one anti-image correlation value or communality below 0.5. We rotated the factors by Varimax rotation. Finally, Fisher's test was used to check for differences in mortality between the wasps previously separated by PCA.

Results

All the behavioural items under study were observed in each aggregation. A few individuals performed most but not all interactions. In particular, the same individuals were present on sugar for a long time, and when they returned to the aggregations other gynes solicited food and sometimes obtained it.

PCA confirmed these observations for each aggregation. From the nine behavioural variables, PCA extracted two PCs in the C and R1 aggregations and three in R2 (for C: KMO = 0.697, explained variance = 86.51%; for R1: KMO = 0.742, explained variance = 75.87%; for R2: KMO = 0.589, explained variance = 78.74%; Bartlett's test: $P < 0.001$ in each case). For each cluster of wasps, one PC was mainly positively represented by trophallaxis given and sugar foraging (PC1 for C and R1, PC3 for R2). With these PCs, we could discriminate a few foraging individuals (helpers) that gave sugar to others (Fig. 1). The "non-foragers" interacted with each other mainly by dominance and food request behaviours; trophallaxis was extremely rare. Three individuals in R1 showed intermediate characteristics and, for this reason, were excluded from the analysis of mortality. Foraging wasps had a higher mortality rate (100%) than other subjects (7.23%) (helpers: 6 died, 0 survived; other gynes: 12 died, 154 survived; Fisher's exact test: $P < 0.001$). The dissection of helpers revealed that they were fertilized.

Discussion

In all the pre-hibernating clusters, a few wasps helped the others by performing external tasks and providing food. During the daily activity, these helpers spent most of their time on the sugar and returned to the aggregation a few times. The short distance to the food and its availability in the cages might have affected the rate of helping behaviour. However, in natural Mediterranean settings, wasps generally aggregate near food resources; thus, the difference in time spent flying to and from them is probably insignificant as compared with the long time spent on food sources. This situation may be limited to populations of warmer temperate zones, in which rich sugar sources are easily available. However, field observations on wasp populations living in different natural settings would be necessary to resolve these issues.

The main question is whether helpers should be identified as gynes or workers. Lack of mating was proposed by O'Donnell (1998) for identifying a worker caste in Vespidae. However, the occurrence of fertilized workers in some *Polistes* species makes O'Donnell's (1998) definition unsuitable for the identification of a worker caste in this genus. Moreover, *Polistes* workers are generally unable to survive winter, yet some workers have been found to hibernate and become foundresses (Reeve *et al*. 1998, Starks 2001). Finally, in some *Polistes* species (including *P. dominulus*) (Turillazzi 1980, Reeve 1991), females do not have morphology- or size-constrained roles. From this perspective, *P. dominulus* seems to be characterized by caste plasticity, in which the roles are highly sensitive to environmental circumstances (Solís & Strassmann 1990, Mead & Gabouriaut 1993, Mead *et al*. 1995). Therefore, it was not possible to characterize helpers as gynes or workers, because even though they helped other reproductive females and died early in winter (like "workers"), they were fertilized (like "gynes").

We found that helping behaviour in aggregations is associated with mortality in the early phase of hibernation. We do not know if the high mortality among helpers is due to the costs of performing the tasks or to some pre-clustering developmental characteristics of helpers. It may be that helpers are weak individuals with reduced possibilities to survive winter and reproduce, and thus with relatively little to lose in helping individuals with higher reproductive potential (West-Eberhard 1975, 2003, Hunt *et al*. 2003).

To obtain some indirect fitness, a helper must direct its efforts towards relatives (Hamilton 1964a, 1964b). As previously hypothesised by Pardi (1942), clustering *P. dominulus* wasps belong to different colonies (Starks 2003). However, philopatry maintains individuals close to their natal nests (reviewed in West-Eberhard 2003), which leads to significant relatedness not only among nestmates but also among individuals living on neighbouring colonies (Ross & Carpenter 1991). As aggregating wasps presumably come from neighbouring nests, they should be fairly closely related to non-nestmate individuals. In this view, the altruistic behaviour of "helpers" would be adaptive in aggregations of *Polistes* wasps that generally show a high population viscosity (Hamilton 1964b, West-Eberhard 1969, 2003, Klahn 1979, Strassmann 1983). Although studies on kin recognition, nesting histories and genetics are necessary to shed light on these issues, helpers might gain some inclusive fitness by helping in aggregations.

In conclusion, pre-hibernating aggregations of *P. dominulus* are not merely associations of individuals living in a group to improve their chance of surviving winter. On the contrary, in this phase, wasps show a network of social interactions and, in particular, a form of division of labour. Certain individuals enter the founding phase with a differential ability to nest alone or to become dominant in polygynic associations (Röseler *et al*. 1984). The events occurring before or during hibernation are good candidates to explain such differences (Reeve 1991).

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