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Nest Inheritance Is the Missing Source of Direct Fitness in a Primitively Eusocial Insect

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Animals that cooperate with nonrelatives represent a challenge to inclusive fitness theory, unless cooperative behavior is shown to provide direct fitness benefits. Inheritance of breeding resources could provide such benefits, but this route to cooperation has been little investigated in the social insects. We show that nest inheritance can explain the presence of unrelated helpers in a classic social insect model, the primitively eusocial wasp *Polistes dominulus.* We found that subordinate helpers produced more direct offspring than lone breeders, some while still subordinate but most after inheriting the dominant position. Thus, while indirect fitness obtained through helping relatives has been the dominant paradigm for understanding eusociality in insects, direct fitness is vital to explain cooperation in *P. dominulus.*

Primitively eusocial species provide a key testing ground for theories of the evolution of sociality, because helpers retain the ancestral ability to breed independently. In insects, such theories focus principally on indirect fitness acquired through aiding genetic relatives, because "sterile" workers in highly eusocial species have limited ability to reproduce (*1*, *2*). However, in the best-studied primitively eusocial system, *Polistes* paper wasps, indirect fitness has failed to fully explain group living (*3*, *4*). *Polistes dominulus* foundresses build new nests in spring, either alone or in small cofoundress groups, and survive for only a single, 5-month-long breeding season. On cofounded nests, subordinates forage to feed the larvae while one dominant individual lays almost all of the eggs (*5*). Inclusive fitness theory thus predicts high relatedness between cofoundresses (*6*), but surprisingly, 15 to 35% of *P. dominulus* subordinates in at least three populations are completely unrelated to the dominant wasp (*3*, *7*, *8*). These unrelated subordinates are the only social insects thought to eschew independent nesting to help raise the offspring of a nonrelative without obtaining inclusive fitness benefits in return (*7*).

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In our study population, 15% of foundresses are unrelated to their cofoundresses, 15% are cousins, and the remainder are full sisters (*9*). The boost to group productivity provided by one subordinate is small enough that even full sisters of the dominant, who share 75% of her genes, would apparently do better to nest alone (*4*, *10*). However, these calculations have not taken into account the direct fitness to be gained from nest inheritance (*7*, *11*). Cofoundress associations are small in *Polistes*, and dominant turnover may be common (*4*, *7*, *12*, *13*), so that subordinates have a chance of inheriting the nest and its work force if the dominant dies or weakens before the breeding season ends (*7*). Indeed, variation in the chance of inheritance seems to drive individual variation in helping effort and aggression in *Polistes* and other primitively eusocial wasps (*12*, *14*, *15*). Studies that have focused on the early breeding season may thus have underestimated subordinate reproductive success [(*16*), but see (*17*)], but the value of inherited resources has rarely been quantified in social insects. Indeed, the relative contribution of direct fitness benefits to social evolution attracts heated debate, even in cooperatively breeding vertebrates where helping by nonrelatives is more common (*18*, *19*).

To evaluate whether subordinates outreproduce lone foundresses even in the absence of indirect fitness benefits, we measured the reproductive success of 1113 foundresses on 228 natural *P. dominulus* nests over the whole nesting season [mean cofoundress relatedness on collected nests = 0.53 ± 0.52 (SEM)]. In early spring 2008, we searched for foundresses that were initiating nests after emergence from hibernation at our study site in southern Spain. Each foundress was marked for identification, and a tarsal sample was taken (*17*) so that any pupae she produced could later be identified by genotyping. Cofoundress group composition fluctuates during the very early spring and stabilizes in late March, at which point we recorded the size of each group and identified the dominant wasp by behavioral censuses (*12*). To confirm the dominant's identity, we also genotyped the first offspring produced (*20*).

Because adult offspring are produced continuously from May until July, it is impossible to estimate reproductive output by sampling at a single time point. Instead, we estimated the number of offspring that subordinates produce in each quarter of the breeding season. First, we collected a random subset of nests at the time when the eggs laid immediately after group stabilization were about to reach adulthood (the early spring collection). We then carried out three more collections, each timed so that the oldest pupae on collected nests had pupated immediately after the previous collection date (late spring, early summer, and midsummer phase collections). We genotyped all pupae on collected nests at nine microsatellite loci, to estimate the number of pupae that subordinates produce in each quarter of the season, for comparison with single foundresses.

Pupae were classified as subordinate offspring if their individual (male) or sibling group (female) genotype was inconsistent with maternity by the dominant wasp but matched that of one or more subordinates. Over half (55.7%) of nests failed through predation or loss of foundresses

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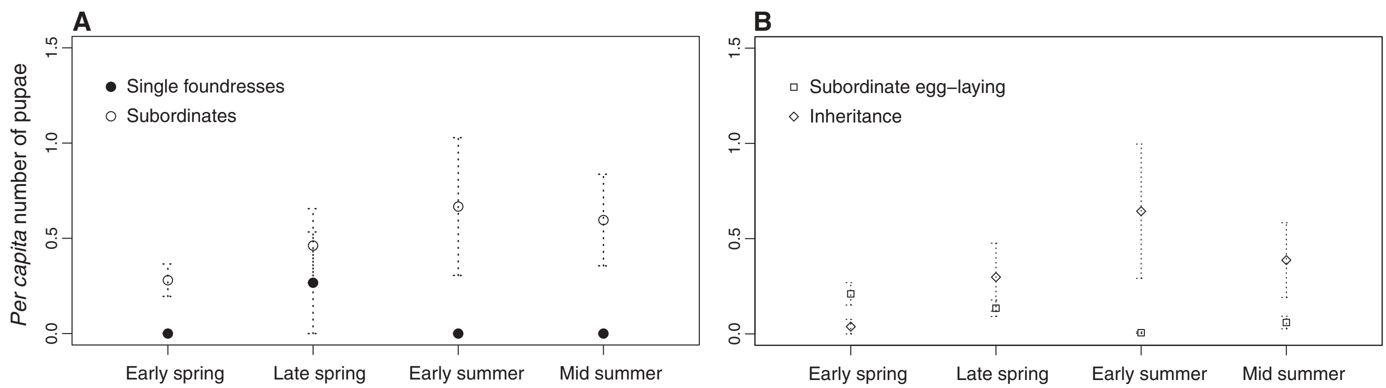

Fig. 1. Mean number of offspring produced by individual subordinates on each nest (per capita) after group stabilization (**A**) compared with lone foundresses (**B**) attained through egg-laying while still subordinate, and through inheriting the dominant position. Error bars mean ± SEM.

before their assigned quarter of the season, and in these cases each subordinate was recorded as having produced no pupae in that quarter. For each nest, we divided the total number of subordinate offspring by the number of subordinate foundresses to obtain subordinate offspring per capita. Remarkably, subordinates produced more direct offspring per capita than lone foundresses [$W = 4980$, $P < 0.001$ (*20*)], because they outreproduced lone foundresses in the latter part of the breeding season (Fig. 1A).

The disparity between lone foundresses and subordinates is greatest in the summer, when offspring are thought to be more likely to become next year's reproductives (*5*). We painted a date-specific mark on all 3072 female offspring that reached adulthood on a separate set of 145 nests, every 6 days from worker emergence until the season ended. In the spring of the following year, the vast majority (90%) of painted foundresses observed initiating spring nests originated from marking dates within the early and midsummer collections from the previous year (Fig. 2). The lone foundresses in our sample failed to produce a single pupa within this period.

Some subordinate offspring (32%) represented eggs laid while the dominant wasp was still alive, but the majority (68%) were produced after the subordinate had inherited the dominant position (Fig. 1B). Inheritance was not observed before the emergence of the nest's first offspring, but occurred most commonly immediately after this period (Table 1), suggesting that subordinates may challenge the dominant when the opportunity to lay offspring that are potential reproductives approaches. Based on the frequency of inheritance in each quarter, we estimate that on 87% of nests, the dominant will retain her position throughout the entire season, so the probability of inheritance for individual subordinates is low. Yet, the high payoff of inheritance, should it occur, means that an average subordinate gains more direct reproduction than an average lone foundress.

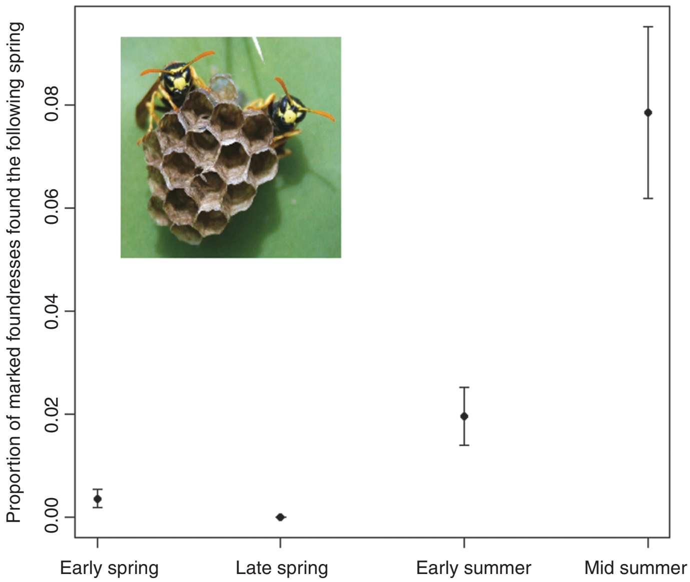

Fig. 2. Proportion of offspring produced during the early spring, late spring, early summer, and midsummer phases that were found on nests the following spring. Error bars, mean ± SEM. **(Inset)** *P. dominulus* cofoundresses on an early spring nest.

Table 1. Inheritance rates in each quarter of the season. Groups of nests were collected at the end of each quarter of the breeding season. If pupal genotypes indicated that a subordinate wasp was dominant at the start of that quarter, inheritance had occurred earlier in the season (column C). If the mother of the oldest pupal group was not the mother of the youngest, inheritance had occurred within that quarter (column D). Approximate dates when pupae were laid as eggs are based on egg/larval development times in (*22*) and pupal development times estimated by monitoring nests from our population, repeated three times over the season.

(A) Collection date (2008)	(B) Breeding season quarter	(C) Nests where inheritance occurred before pupal broods were produced (%)	(D) Nests where inheritance was observed within the pupal brood (%)
18 May	**Mid spring.** Pupae represent eggs laid before early May.	0	0
16 June	**Late spring.** Pupae represent eggs laid throughout May.	0	11.32
3 July	**Early summer.** Pupae represent eggs laid in early and mid-June.	9.76*	2.44
15 July	**Late summer.** Pupae represent eggs laid in late June and early July.	7.41	0

*One nest where the original dominant reinherited after the death of a usurper was not included in this statistic.

When inheritance occurs, the payoff to the inheritee is greater in larger cofoundress groups, because such nests are less prone to failure through predation or foundress death, especially early in the season (group size/collection date interaction: $\chi^2 = 7.65$, df = 1, $P < 0.01$) (fig. S1A). Furthermore, when they survive, nests founded by larger groups produce more pupae than their smaller surviving counterparts ($F_{1,96} = 13.0$, $P < 0.001$) (fig. S1B). However, in these larger cofoundress groups, each individual subordinate stands a lower chance of inheriting, because she faces greater competition from nestmates. Accordingly, subordinates produced the most offspring through inheritance per capita in medium-sized cofoundress groups (Fig. 3). However, we found no evidence that unrelated subordinates preferentially joined medium-sized groups (group size/relatedness correlation, Spearman's Rho: $P = 0.86$).

As well as sometimes inheriting the nest, foundresses could lay eggs while still subordinate. Subordinate egg-laying was more common in larger cofoundress groups ($F_{1,178} = 10.62$, $P < 0.01$) (Fig. 3). This might reflect enhanced difficulties in policing subordinate reproduction, or dominants in larger groups might allow more subordinate egg-laying to reduce the incentive for subordinates to fight for control of highly productive nests ("peace incentives") (*6*, *14*, *16*). Although subordinate egg-laying represented a smaller proportion (32%) of subordinate direct fitness than reproduction through inheritance (68%), even subordinates that do not inherit the dominant position could equal or exceed the reproductive output of lone foundresses (per capita reproduction through subordinate egg-laying versus lone foundress reproduction; $W = 3326$, $P < 0.01$) (Fig. 3).

Our analysis focused on average per capita subordinate reproduction, but it is possible that unrelated individuals (16.9% of subordinates in our sample) achieve less direct reproduction than others. To investigate this, we compared the per capita number of female pupae produced by subordinates that were relatives of the dominant (sisters or cousins), or were unrelated to her, across all nests that survived until collection. Overall, there were no significant differences ($P = 0.37$). We also found no relationship between mean cofoundress relatedness and the total productivity of the nest ($F_{1,84} = 1.73$, $P = 0.19$).

Our findings explain why only 4.04% of wasps chose to remain as lone foundresses at group stabilization (fig. S2): Individuals can

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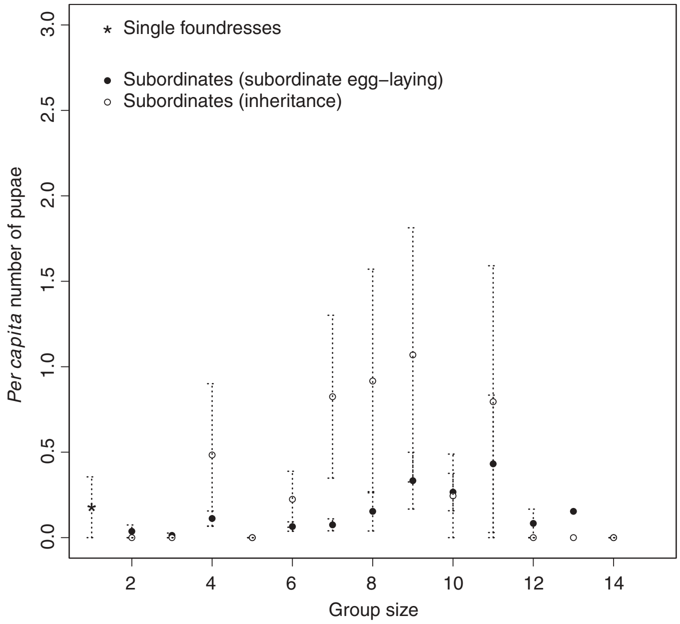

Fig. 3. Subordinate reproductive prospects, through subordinate egg-laying and inheritance, according to group size. Lone foundresses are illustrated for comparison. Error bars, mean ± SEM.

achieve more direct fitness through subordination even to a nonrelative than through nesting alone. However, we do not imply that direct fitness benefits are always the main driver of subordinate behavior, because our data also show that indirect benefits usually outweigh direct benefits for those subordinates [~56 to 70% (*7*, *9*)] that are relatives of the dominant wasp (fig. S3). Rather, direct fitness benefits make subordination worthwhile if wasps either do not have surviving relatives in the population or fail to recognize them. Within our sample, at least 12.8% of unrelated subordinates had sisters that were dominant on nearby nests, suggesting that kin recognition sometimes fails. Individuals should choose to nest with their sisters where possible, but the prospect of nest inheritance means that subordination can be adaptive even when this ideal cannot be achieved.

The importance of inheritance for *P. dominulus* subordinates, even within their short nesting season, means that like helpers in cooperatively breeding vertebrates, their behavior must reflect a trade-off between current (indirect) and future (direct) fitness (*12*, *14*). Inheritance has the potential to stabilize cooperation, because a dominant cannot easily accept help from subordinates, then later renege on the inheritance payoff after her own death (*21*). However, subordinate reproduction will also reduce relatedness between workers and egg-laying foundresses later in the season, helping to explain why a committed altruistic caste has not evolved in *Polistes* (*1*).

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Supporting Online Material

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Materials and Methods
Figs. S1 to S5
Tables S1 and S2
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Archaeorhizomycetes: Unearthing an Ancient Class of Ubiquitous Soil Fungi

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Estimates suggest that only one-tenth of the true fungal diversity has been described. Among numerous fungal lineages known only from environmental DNA sequences, Soil Clone Group 1 is the most ubiquitous. These globally distributed fungi may dominate below-ground fungal communities, but their placement in the fungal tree of life has been uncertain. Here, we report cultures of this group and describe the class, Archaeorhizomycetes, phylogenetically placed within subphylum Taphrinomycotina in the Ascomycota. Archaeorhizomycetes comprises hundreds of cryptically reproducing filamentous species that do not form recognizable mycorrhizal structures and have saprotrophic potential, yet are omnipresent in roots and rhizosphere soil and show ecosystem and host root habitat specificity.

Direct sequencing of environmental DNA is a powerful tool to explore cryptic diversity of microorganisms and challenges our understanding of global biodiversity (*1*, *2*). Despite producing macroscopic reproductive structures and being among the largest of eukaryotes (*3*), many fungal species and even phyla have seldom been observed or cultivated (*4*–*6*). Among the lineages known only from environmental DNA sequences, the Soil Clone Group 1 (SCG1) (*5*) is the most common enigmatic lineage in soil (*7*, *8*). The mysterious nature of SCG1 stems from its detection by sequencing in more than 50 ecological studies of soil fungi (tables S1 and S2), but the organisms have never before been observed in the form of fruiting body, spore, culture, or distinctive

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