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ORIGINAL PAPER

Cofoundress relatedness and group productivity in colonies of social *Dunatothrips* (Insecta: Thysanoptera) on Australian *Acacia*

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Abstract Facultative joint colony founding by social insects provides opportunities to analyze the roles of genetic and ecological factors in the evolution of cooperation. Although cooperative nesting is observed in range of social insect taxa, the most detailed studies of this behavior have been conducted with Hymenoptera (ants, bees, and wasps). Here, we show that foundress associations in the haplodiploid social thrips *Dunatothrips aneurae* (Insecta: Thysanoptera) are most often comprised of close relatives (sisters), though groups with unrelated foundresses are also found. Associations among relatives appear to be facilitated by limited female dispersal, which results in viscous population structure. In addition, we found that per capita productivity declined with increasing group size, sex ratios were female-biased, and some female offspring apparently remained in their natal domicile for some time following eclosion. *D. aneurae* thus exhibits a suite of similarities with eusocial Hymenoptera, providing evidence for the convergent evolution of associated social and life-history traits in Hymenoptera and Thysanoptera.

Keywords Pleometrosis · Evolution of cooperation · Sex ratio · Social evolution · Life history

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Introduction

Explaining conditions that promote the evolution of sociality and cooperation is fundamental to understanding many of the major transitions in evolutionary history (Maynard Smith and Szathmary 1995) and continues to be a challenge for evolutionary biologists. The costs and benefits of social behavior are ultimately determined by ecological factors, but the degree to which the benefits must exceed the costs depends critically on the genetic relatedness of interactants. As originally demonstrated by Hamilton (1964), individuals can increase the indirect component of their inclusive fitness by directing cooperative actions toward relatives. High genetic relatedness between individuals partially offsets the costs of cooperating, meaning that the behavior can be favored by natural selection even when the benefit to cost ratio is relatively low. Although high genetic relatedness among group members tends to facilitate the evolution of cooperation, it is by no means necessary, as cooperation between unrelated individuals is also observed (Clutton-Brock 2002; Lehmann and Keller 2006). In such cases, cooperating individuals typically benefit directly through eventual gains in personal reproduction or survival.

Joint colony founding by social insects provides an arena for investigating the relative contributions of genetic and ecological factors to the evolution of cooperation. Although nests of most social insects are established independently by a single female (haplometrosis), colony founding by multiple females (pleometrosis) occurs in a number of social insect groups including ants (Herbers 1993; Choe and Perman 1997; Cahan et al. 1998; Bernasconi and Strassmann 1999; Johnson 2004), bees (Kukuk and Sage 1994; Schwarz et al. 1997; Schwarz et al. 1998), wasps (Reeve 1991; Ito 1993), termites (Shellman-Reeve 1997; Hacker et al. 2005), mites (Saito 1997), aphids (Miller 1998a, b, 2004), and thrips

(Morris et al. 2002; Crespi et al. 2004; Bono and Crespi 2006).

Cofounding has been studied most extensively in the ants, bees, and wasps, and the relative importance of genetic and ecological factors varies considerably for different species. Cooperating foundresses are generally unrelated in ants (Bernasconi and Strassmann 1999) and some bees (Kukuk and Sage 1994; Danforth et al. 1996; Paxton et al. 1996); such cofoundress associations are usually communal in that reproduction is shared more or less equally among females, with low reproductive skew (Kukuk and Sage 1994; Crespi and Choe 1997; Reeve and Keller 2001). Thus, per capita brood production either increases or remains constant with increasing numbers of foundresses (Kukuk and Sage 1994; Danforth et al. 1996; Crespi and Choe 1997), though there are some exceptions, particularly in ants (Bernasconi and Strassmann 1999). In contrast, cofoundresses are typically related in eusocial wasps and bees (McCorquodale 1988; Reeve 1991; Kukuk and Sage 1994; Crespi and Choe 1997; Schwarz et al. 1997; Reeve and Keller 2001), though more recent evidence suggests that the presence of unrelated females may be more common than previously assumed (Queller et al. 2000; Fanelli et al. 2005; Liebert and Starks 2006; Nonacs et al. 2006). In either case, production of workers and reproductives is often dominated by a single female (high reproductive skew) (Reeve 1991; Reeve and Keller 2001; Liebert and Starks 2006) and per capita brood production declines with increasing group size (Michener 1964; Karsai and Wenzel 1998; Clouse 2001; Soucy et al. 2003; Liebert and Starks 2006), though there are some exceptions to this general pattern (Schwarz et al. 1998; Bouwma et al. 2006; Smith et al. 2007). The relative importance of core social traits including relatedness, ecological context, per capita reproduction, group size, and life history in animals with foundress associations is a key, unresolved question in behavioral and evolutionary ecology (Crespi and Choe 1997), with important implications for how natural selection shapes different forms of sociality.

The recent discovery of cofounding of colonies in a genus of Australian *Acacia* thrips provides a novel system for analyzing the evolutionary dynamics of sociality, relatedness, cooperation, and life history (Morris et al. 2002; Crespi et al. 2004; Bono and Crespi 2006). Several species in the genus *Dunatothrips* construct domiciles on their host plants by joining together phyllodes (leaf-like extensions of the petiole) using a silk-like secretion to form an enclosed space for feeding and breeding.

This study focuses on *Dunatothrips aneurae*, which is distributed throughout the arid regions of South Australia, New South Wales, Northern Territory, and Queensland, Australia, where it constructs domiciles on its host plant *Acacia aneura*. Like other *Dunatothrips*, *D. aneurae* foundresses in newly constructed domiciles are always dealated (have shed their wings), though it is not known when dealation occurs. Females remain within the domicile after construction is completed, feeding on plant cell contents and producing a brood cohort that develops to adulthood inside. A recent study on *D. aneurae* indicated that joint nesting provides benefits to group members in terms of increased foundress survival and protection of brood against kleptoparasites while also resulting in lower average individual productivity (Bono and Crespi 2006). Associations form before domicile construction is complete, but the behavioral aspects of colony foundation (such as shared building), the relatedness of cofoundresses, and the degree of reproductive skew within colonies have yet to be investigated.

In this study, we use microsatellite markers to provide the first data on patterns of relatedness among *D. aneurae* cofoundresses. Furthermore, we use a new dataset to replicate the previous finding (Bono and Crespi 2006) that per capita brood production declines with group size. We also use census data to show that females do not appear to move between domiciles after domicile construction is complete, that some female offspring may shed their wings and remain in their natal domicile for some period of time after eclosion, and that offspring sex ratios are female-biased. Finally, we synthesize these findings using comparisons of patterns in relatedness, sex ratio, brood production, and life history in *Dunatothrips* with those of other social insects.

Materials and methods

Collections

Colony founding in *D. aneurae* occurs throughout the year, but founding events appear to be synchronized, possibly in response to environmental cues such as rainfall (domiciles are usually constructed on newer phyllodes that begin growing following the rain). Thus, a single tree can contain colonies at different stages of development that are organized into distinct cohorts. Data that we report in this study come from two collections of *D. aneurae* colonies. In March 2005, we collected a total of 348 colonies at various stages of development from seven locations in South Australia and New South Wales, Australia. More details on this collection are provided elsewhere (Bono and Crespi 2006). In September 2005, we collected a total of 503 *D. aneurae* colonies from four sites near Broken Hill, NSW, Australia (Appendix 1). Rainfall prior to this collecting trip resulted in abundant new growth on the host plant, *A. aneura*, and *D. aneurae* colonies were much more common than on the previous trip. Additionally, in contrast to the earlier collection, nearly all colonies in this sample were at an early stage of development. Domiciles were removed from the host plant intact and transported to the laboratory where they were either stored in a 20% dimethylsulfoxide solution saturated with salt or frozen for genetic analysis.

Genetic analysis

For genetic analyses, we used primers designed to amplify two trinucleotide microsatellite repeats (Dunato 1 and Dunato 2) and one tetranucleotide microsatellite repeat (Dunato 3) that were isolated by Genetic Identification Services (Chatsworth, CA, USA). Primer sequences are given in Appendix 2. We extracted DNA from thrips (after removing the bottom third of the body to avoid possible sperm contamination) using the AquaPure Genomic DNA tissue kit from Bio-Rad. We amplified gene products with the polymerase chain reaction using the appropriate annealing temperature (Appendix 2). We separated products on 6% polyacrylamide gels using a LICOR 4300 DNA analyzer. Genotypes were scored with the aid of the computer software package Gene ImageIR version 4.05 (Scanalytics), which scores bands based on their relative migration compared to standards of known size. To further verify the accuracy of allele scoring, we ran samples from different gels that were deemed to be of the same size next to each other on a subsequent gel. We genotyped a total of 91 females from 29 different colonies collected from site three. All of these colonies had either no brood or only eggs, so we are confident that the females we genotyped were foundresses and not offspring of the original foundresses (see below). The colonies were distributed on two trees that were spaced approximately 120 m apart, with 20 coming from the first tree and the other nine coming from the second.

Foundress relatedness and population structure

We used two methods to examine genetic structure in our dataset. First, we used an analysis of molecular variance (AMOVA) implemented in the computer program GeneAlEx 6 (Peakall and Smouse 2006) to estimate hierarchical genetic structure using four levels: individual (I), domicile (D), tree (S), and total population (T). We report three F statistics from this analysis: F_{ST}, F_{DS}, and F_{ID}. In order to calculate F_{IS}, we created 20 simulated datasets by randomly selecting one individual from each domicile (otherwise data points would not be independent). For each simulated dataset, we used the computer program FSTAT 2.9.3 (Goudet 1995) to calculate F_{IS}; the value that we report is the mean of these 20 datasets. We only report this statistic for one of the two trees because sampled domiciles from the excluded tree belonged to one of only three family groups, meaning that even individual samples from different domiciles were not independent samples. Statistical significance of F_{ST}, F_{DS}, and F_{ID} was determined using the permutation procedure (1,000 permutations) available in GeneAlEx 6. While a significant F_{ST} in this analysis indicates that cofoundresses associate nonrandomly with respect to relatedness, group relatedness values and pairwise relatedness values are not estimated. We thus used the computer program Relatedness 5.0.8 (K. F. Goodnight; http://www.gsoftnet.us/GSoft.html) to calculate these quantities. Because the AMOVA analysis revealed significant genetic structure between trees, we performed the relatedness analysis separately for each tree (i.e., allele frequencies used were calculated separately from each tree). We weighted individuals equally in these analyses and a bias correction using the group variable was applied. Standard errors for average relatedness of groups within the entire population and of groups on each local population were obtained by jacknifing over groups, while standard errors for individual group estimates were obtained by jacknifing over loci.

Given the relatedness results (see below), we were interested in whether some cofoundresses on tree one were unrelated. To investigate this possibility, we used the computer program Kinship 1.3 (http://www.gsoftnet.us/GSoft.html) to generate relatedness values for 2,000 simulated pairs of haplodiploid full sisters (r=0.75), female cousins (r=0.1875), or unrelated females (r=0) using the observed allele frequencies from the relatedness analysis. Given what we know about the life history of *D. aneurae*, these putative relationships seem most likely, though other possibilities such as half sibs are also possible. For each relationship, we sorted the simulated pairs into relatedness bins ranging from 1 to −1 (intervals were 0.1), which allowed us to calculate a frequency for each bin given the specific relationship. Given this, we then performed an analysis that calculated likelihoods that our observed distribution was comprised of specific frequency mixtures of sisters, cousins, and nonrelatives (e.g., 40% sisters, 40% cousins, 20% unrelated). This allowed us to select the frequency mixture with the highest likelihood relative to all other scenarios.

Movement between domiciles and retention of offspring in the natal domicile

Observations of colony demographics in this study suggested that the number of dealates might be higher in mature colonies than in immature colonies. This could occur for two reasons: (1) dealate females could move between domiciles (or newly arriving females could join domiciles), even after domicile construction is complete, or (2) some offspring could shed their wings and remain in the domicile following eclosion. To test these alternatives, we categorized colonies from the March 2005 collections (the September 2005 collections did not include mature colonies) into three developmental stages (stage 1, foundresses with no brood; stage 2, foundresses with developing brood; stage 3, alate adult brood) and used an ANOVA to compare the number of dealate females present in colonies at these stages. Although these data do not allow us to fully differentiate between the two hypotheses presented above, a pattern showing a spike in dealate numbers restricted to the time of brood emergence would give more support for hypothesis two, because there is no reason to expect movement of dealates to occur only during a narrow window of time following brood emergence. Moreover, while a linear increase in dealate numbers over stages 1 and 2 would fully support hypothesis one, it would not be possible to rule out hypothesis two if the increase was also seen during stage 3. We also used a chi-square test to compare the proportion of colonies with more than one dealate female for immature colonies (stages 1 and 2) and mature colonies (stage 3). We included both live and dead dealates in both tests so as to ensure that any survival differences between dealates in single vs. multiple foundress colonies did not bias our results.

Brood production and sex ratio

We used the September 2005 dataset to attempt to replicate the previous finding that per capita brood production declined with increasing foundress numbers (Bono and Crespi 2006). Specifically, we used least squares regression to analyze the relationship between foundress number and per capita brood production. To assess whether a nonlinear function better fit the data, we compared a model using a quadratic term with a model using a standard straight-line fit. All colonies included in this analysis were at an early stage of development, having only eggs and/or larvae. Thus, we are confident that dealates in these nests were foundresses and not recently eclosed offspring. We excluded colonies in which females had not started laying eggs. We calculated numerical sex ratios (proportion of males (p_{m})) for colonies with adult brood (developing brood could not be sexed). Only alate adults were counted in these calculations because we could not determine whether dealate females were foundresses or offspring that had shed their wings. These calculations were made from an average of five individuals per nest.

Results

Of the 503 colonies that we collected in September 2005, 40% were initiated by multiple females (range, 1–12; Fig. 1). Overall, 68% of foundresses were involved in associations. Information on the March 2005 collection is reported in Bono and Crespi (2006).

Foundress relatedness and population structure

The AMOVA analysis indicated significant genetic structure between domiciles on a tree and between trees (Table 1). Significant structure between domiciles suggests that cofoundresses are sometimes relatives, and indeed, the relatedness analysis revealed that average relatedness among nestmate foundresses was relatively high (0.452, confidence interval (CI), 0.193). However, this value varied substantially for the two trees (tree one—0.198, CI, 0.114; range, −0.581–0.658; tree two—0.849, CI, 0.417; range, 0.822–1.00). While it is clear that cofoundresses on tree two were generally highly related, the lower mean and wider range on tree one prompted us to investigate whether some cofoundresses on this tree were unrelated. The distribution of individual pairwise relatedness estimates showed peaks at 1 and 0.75, which was similar to the distribution for simulated full sisters, and also a series of peaks falling within the range of the distributions for cousins and unrelated females (Fig. 2). The likelihood analysis suggested that associations on this tree included sisters and unrelated females as the maximum likelihood was achieved when 32% were drawn from the full sister distribution and 68% were drawn from the unrelated distribution (Fig. 3). Considering the fact that other scenarios, some involving cousins, also had relatively high likelihoods, we do not place great confidence in these precise figures. However, this analysis does strongly suggest that cofoundresses on this tree represented some mix of related and unrelated females. Indeed, the maximum likelihood scenario was at least 9.5×10^{17} times more likely than any scenario with no relatives. Likewise, the maximum likelihood scenario was at least 10,000 times more likely than any scenario with no unrelated females and at least 50 times more likely than any scenario with fewer than 25% unrelated females.

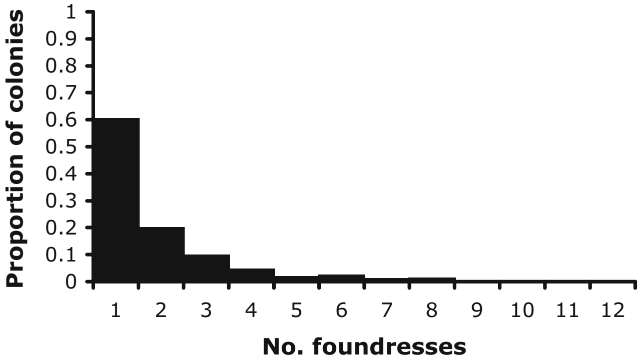


Fig. 1 Distribution of foundress numbers in *D. aneurae* colonies collected in September 2005 (N=503)

Table 1 AMOVA summary table showing the percentage of molecular variance observed at each hierarchical level, *F* statistics, and their statistical significance

Level	% of variance	*F* statistic	*P* value
F_{ST}	12	0.125	<0.001
F_{DS}	23	0.259	<0.001
F_{ID}	65	0.351	<0.001

Because the AMOVA analysis revealed significant genetic structure between trees that were in relatively close proximity (~120 m apart), we further examined genotypes in order to determine whether there was evidence for limited dispersal through colony budding. Visual inspection of genotypes for tree two suggested that females in these nine colonies probably belonged to one of the three simple family groups. A total of 11 females belonging to four different domiciles had genotypes consistent with them being full sisters, as all shared one allele (inferred as the paternal allele) and at least one other allele. We therefore grouped these putative relatives together in a separate analysis and relatedness was high (*r* when all combined= 0.903, CI, 0.317). An additional four domiciles (total of 13 females) contained another set of apparent close relatives (*r* when all combined=0.872, CI, 0.562). Finally, a third colony comprised of two females (*r*=0.832, CI, 1.016) was not related to any other domiciles sampled. Our interpretation of these data is that some females do not disperse far from their natal nest, instead establishing new domiciles with former nestmates in close proximity to the original nest. The mean F_{IS} from the 20 simulated datasets was low and the 95% confidence interval overlapped zero (F_{IS}= 0.023 [−0.010, 0.056]), suggesting minimal inbreeding (a formal test of significance was not available in FSTAT).

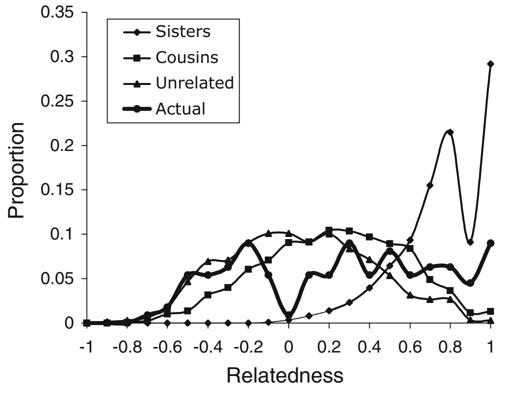


Fig. 2 Observed relatedness distribution for *D. aneurae* cofoundresses on tree one and expected distributions for full sisters (true *r*= 0.75), cousins (true *r*=0.19), and unrelated females (true *r*=0). Expected distributions were generated using the simulation option in the computer program Kinship

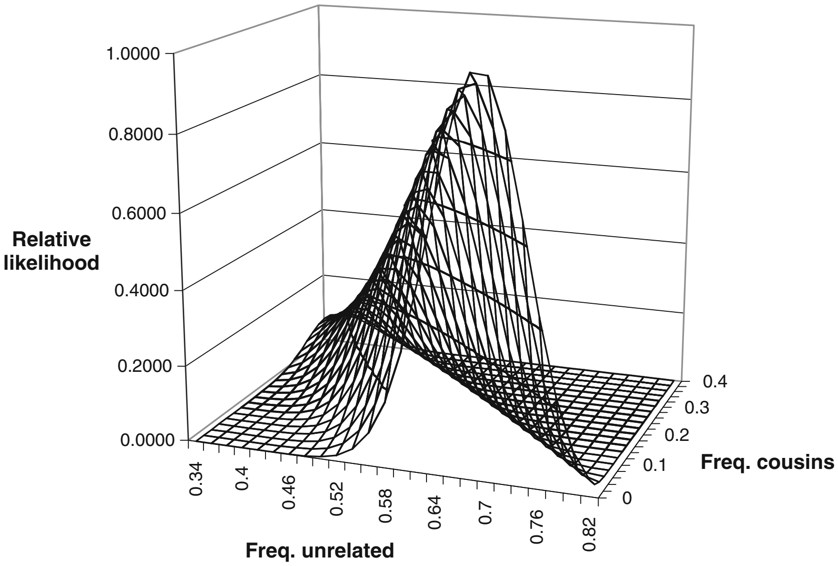
Movement between domiciles and retention of offspring in the natal domicile

A one-way ANOVA revealed differences in the number of dealates present in colonies at different stages of development (one-way ANOVA, $F_{2,181}$=8.2, *P*=0.0004). A further comparison of individual means showed that the observed difference was because colonies at stage 3 had more dealates than those at stage 1 or 2, which were not different from each other (Tukey's honestly significant differences (HSD), α=0.05; Fig. 4). These results thus provide circumstantial evidence that some offspring shed their wings upon eclosion, unless movement between domiciles is also restricted to the narrow period of time following brood emergence. Additional support for the hypothesis that some females shed their wings and remain in the domicile comes from the fact that a higher proportion of colonies at stage 3 had more than one dealate (71%) compared with that for colonies at stage 1 (27%) and 2 (24%) (Pearson chi square, $\chi^2_{181} = 28.5$, *P*<0.001; Fig. 4). An alternative interpretation of these data is that colonies with more dealate foundresses initially were overrepresented in the stage 3 samples because of survivorship advantages. However, our analysis included both live and dead individuals, meaning that, for this to be true, the entire domicile and all of its contents would have had to disappear in a short period of time. We find this to be unlikely given that we routinely find domiciles with dead foundresses.

Brood production and sex ratio

The regression analysis revealed a significant negative relationship between foundress number and per capita brood production (least squares regression, $F_{1,304}$=40.6, *P*<0.0001, R^2=11.8%). Nevertheless, a model using a quadratic term produced a better fit, albeit with more parameters, suggesting that the decline in per capita reproduction begins to level off somewhat at approximately five foundresses (least squares regression, $F_{2,303}$=25.4, *P*<0.0001, R^2=15.2%; Fig. 5). One of the potential problems with this analysis is that all colonies with at least one egg were included in the analysis (colonies with no eggs were excluded). Thus, one alternative explanation for the decline in per capita reproduction with increasing foundress number could be that foundresses in associations take longer to begin laying eggs, which might lead to a spurious negative correlation between per capita productivity and the number of foundresses. If this were true, then we would expect multiple foundress colonies to be overrepresented in the group that was excluded from the analysis relative to the colonies being analyzed. In fact, this is not the case, as there was no difference in the frequency of multiple foundress colonies in either group (42% for

Fig. 3 Surface plot showing the relative likelihood of various combinations of full sisters, cousins, and unrelated females, given the observed distribution of relatedness estimated on tree one. The maximum likelihood was achieved when 32% were drawn from the full sister distribution and 68% were drawn from the unrelated distribution



colonies with brood vs. 36% for colonies with no brood, Pearson chi square, $\chi^2_{500} = 2.16$, P=0.14).

Overall, brood sex ratios were highly female-biased (overall proportion of males, p_m=0.290; chi-square goodness of fit test, $\chi^2_{307} = 27.1$, P<0.001; Table 2). Average colony sex ratios were similarly female-biased (Table 2) and there was no evidence for split sex ratios, as only 14% of colonies produced single sex broods. No colonies produced exclusively of male brood, meaning that at least one female in all colonies had mated. Our sex ratio results have two caveats. First, males are sometimes present during colony founding and are thus probably not offspring of any of the foundresses. Because we had no way to distinguish these males from offspring, they may have been included in calculations. Second, we did not count dealate females in our computations; although some of these females may have been offspring of the original foundresses, we cannot differentiate between these females and the actual foundresses. In either case, the result would be an underestimate of female allocation, meaning that our female-biased results are probably conservative estimates.

Discussion

Average group relatedness in foundress associations of *D. aneurae* across all groups was high (r=0.45), but pairwise estimates demonstrated that whereas many associations were apparently comprised of full sisters, some associations, concentrated on one tree, involved unrelated females. This tree had a notably large number of foundresses initiating colonies, which raises the possibility that the frequency of associations among nonrelatives is in some way linked to high foundress densities.

Our results parallel those from recent studies on some social bees and wasps, which have shown that although most

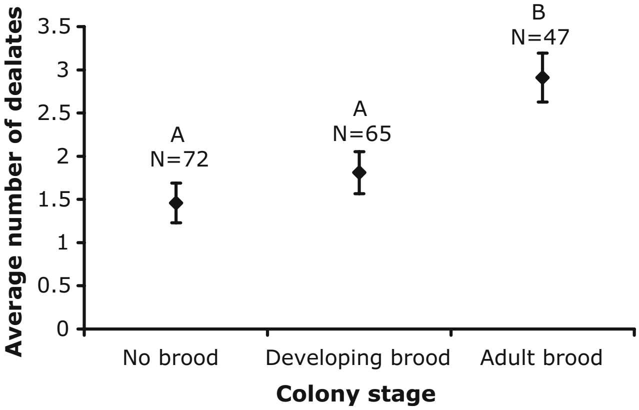


Fig. 4 Mean number of dealated females (*error bars* represent standard errors) present in colonies at different stages of development. Groups labeled with different *letters* indicating those that were statistically significant (Tukey's HSD, P<0.05)

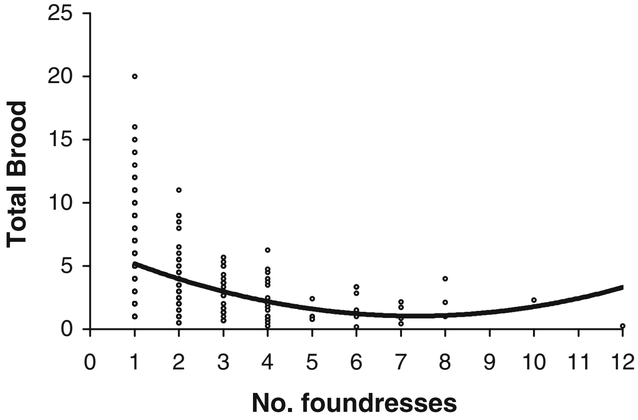


Fig. 5 The relationship between foundress number and per capita brood production

Table 2 Population numerical sex ratios (proportion of males, p_m) and mean colony sex ratios for *D. aneurae* colonies with adult brood

Site	Population p_m (*N*)	Mean colony p_m±standard errors (*N*)
6	0.318 (22)	0.214±0.127 (4)
7	0.278 (194)	0.297±0.050 (23)
8	0.257 (35)	0.253±0.086 (8)
9	0.333 (12)	0.313±0.188 (4)
10	0.231 (26)	0.181±0.066 (7)
11	0.500 (18)	0.511±0.146 (3)

cofounding females are close relatives, groups with unrelated females are common (Queller et al. 2000; Fanelli et al. 2005; Liebert and Starks 2006; Nonacs et al. 2006). In some of these cases, unrelated females appear to gain direct benefits. For example, Queller et al. (2000) demonstrated that unrelated *Polistes dominulus* subordinates benefit from the possibility of inheriting dominant status in the event that the original dominant female dies. Likewise, unrelated cofoundresses of the allodapine bee, *Exoneura robusta*, may gain direct benefits because per capita reproduction increases with group size and reproductive skew tends to be low (Schwarz and O'keefe 1991; Silberbauer and Schwarz 1995; Langer et al. 2006). Alternatively, groups comprised of nonrelatives or those with a subset of unrelated individuals may represent suboptimal associations that result from recognition errors (Nonacs et al. 2006). These recent discoveries in Hymenoptera, and our study of social *Dunatothrips*, demonstrate that theories addressing the selective causes of cofoundress associations in social animals must explicitly account for associations that involve both close relatives and nonrelatives, even in the same populations.

We have also demonstrated in this study that *D. aneurae* females in cofoundress associations do not gain direct fitness through enhanced individual productivity. In fact, per capita reproduction declined with group size, indicating that reproductive output is actually lower for at least some females in such groups (see also Bono and Crespi 2006). Per capita reduction in productivity does not, however, necessarily preclude direct benefits to cofounding because higher offspring survival, or an increased probability of nesting success in multiple female colonies, could compensate for lower individual productivity provided that all individuals reproduce to some degree. Indeed, results of an earlier study suggested that brood survival might be enhanced in multiple female *D. aneurae* colonies (Bono and Crespi 2006).

The combined pattern of high foundress relatedness in most groups and declining individual productivity with increasing group size is common for facultatively eusocial Hymenoptera with reproductive division of labor (Crespi and Choe 1997). The generality of this relationship across social animals is difficult to assess given the paucity of data on relatedness in foundress associations of non-Hymenopterans. However, some Hymenoptera with apparently communal nests may exhibit high relatedness among nestmates (McCorquodale 1988), and in social groups of some larval Lepidoptera and social spiders, reproductive division of labor does not occur even when relatedness among associates is high (Aviles 1997; Uetz and Hieber 1997). These comparative patterns suggest that the association between relatedness and reproductive skew is strongly modulated by aspects of ecology and demography that may differ fundamentally between groups of social animals, such as factory-fortress species (whose domicile provides both food and shelter), 'life insurers' (that must perform risky tasks such as foraging outside the domicile), and vertebrates with long life spans (Strassmann and Queller 1989; Crespi 2007). Ongoing studies aimed at estimating the relative reproductive contributions of different females are crucial to evaluating the status of *D. aneurae* as either a communal breeder or a cooperative breeder with reproductive division of labor.

The fact that many *D. aneurae* cofoundresses are sisters implies that females have some mechanism for finding relatives during colony initiation. As Hamilton (1964) suggested, associations among relatives could theoretically arise by two mechanisms: (1) individuals may actively discriminate between relatives and nonrelatives, or (2) limited dispersal may generate 'viscous' population structure such that local neighborhoods are generally comprised of close relatives, thereby allowing cooperative behaviors to be dispensed without any discrimination per se. Although we currently do not have enough information to differentiate between these two mechanisms, considerable genetic structure over a limited spatial scale coupled with the identification of several closely related *D. aneurae* colonies on the same tree implies that at least some females remain in the vicinity of their natal nest and begin forming new colonies with former nestmates. If some females leave their natal domicile on foot with other nestmates, active discrimination between relatives and nonrelatives may not be necessary under normal circumstances.

Two additional observations from our study have important implications for understanding the life history and social system of *Dunatothrips aneurae* and comparing this species with more 'traditional' social insects. First, not only do foundresses shed their wings prior to establishing a domicile, but some first-generation female offspring appear to shed their wings and remain in the natal domicile for some period of time following eclosion. This pattern of apparent commitment to the natal domicile, and subsequent generation overlap, resembles the recruitment of offspring as workers in social Hymenoptera, although the nature of any 'work' performed by such offspring, other than expansion of the domicile and possibly colony defense, remains unclear. Second, we observed highly female-biased sex ratios, a pattern that has long been associated with eusociality in Hymenoptera because females are morphologically and behaviorally specialized for helping, and haplodiploid female helpers may in some circumstances gain more inclusive fitness through rearing sisters than brothers (Bourke and Franks 1995). In *D. aneurae*, females are larger and exhibit more-armed forelegs than males (personal observation), so a female bias to any helping behavior might be possible. Overproduction of females may also be explained by local mate competition (Hamilton 1967), which has been documented in multiple species of social gall-inducing thrips (Crespi et al. 2004). However, local mate competition is generally associated with inbreeding, for which we found no evidence, though it is theoretically possible that related males could compete for mates without inbreeding. Another possibility is that our sex ratio data could be biased. If, for example, females typically emerge earlier than males (protogyny), then our calculations could be unreliable as many colonies still had developing brood at the time of census. Future detailed work on the mating system, colony development, and individual behavior are necessary to differentiate among these possibilities.

Taken together, the results of our study indicate that *D. aneurae* exhibit a suite of similarities with eusocial Hymenoptera, including cofoundress associations comprised of close relatives (with some mixing of nonrelatives), domicile construction, dealation of foundresses, haplodiploidy, female-biased sex ratios, per capita reductions in productivity with foundress number, and retention of first-generation offspring within the expanding domicile. The convergent evolution of this suite of traits in Hymenoptera and Thysanoptera is suggestive of common selective pressures, which may be potentiated in part by haplodiploid reproduction. Additional studies of the genus *Dunatothrips* should thus provide fertile ground for future research on the costs and benefits of cooperative nesting.

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Appendix 1

Table 3 Summary of *D. aneurae* colony samples from two collecting trips to New South Wales (NSW) and South Australia (SA)

	Site (state)	Number of trees with *Dunatothrips*	Number of colonies collected
September 2005	1. S 31° 56.241′ E 141° 30.259′ (NSW)	2	60
	2. S 31° 55.747′ E 141° 31.569′ (NSW)	10	114
	3. S 31° 56.060′ E 141° 27.601′ (NSW)	3	249
	4. S 31° 55.669′ E 141° 29.294′ (NSW)	2	80
March 2005	5. S 31° 16.766′ E 143° 11.668′ (NSW)	1	3
	6. S 31° 12.272′ E 143° 10.845′ (NSW)	5	13
	7. S 31° 01.030′ E 142° 16.879′ (NSW)	1	130
	8. S 31° 17.613′ E 142° 10.638′ (NSW)	4	67
	9. S 31° 30.571′ E 137° 06.314′ (SA)	3	53
	10. S 30° 56.594′ E 135° 43.848′ (SA)	3	52
	11. S 30° 58.164′ E 135° 45.119′ (SA)	2	30

Appendix 2

Table 4 Primer sequences, number of alleles, H_o (observed heterozygosity), and allele size ranges for three microsatellites used to analyze cofoundress relatedness

Locus	Repeat motif	Primers sequences (5′–3′)	Number of alleles	H_o	Allele size range
Dunato 1	CAA	GCGTGTGGTATTATTGTTGAC CCCAGAGGCAGAGCATAT	8	0.65	201–231
Dunato 2	CAA	TTGATCAAAATTGCGACGTT TAAAACCGCCCAACTACTCG	6	0.59	160–205
Dunato 3	GTTT	GCGGTGGTCTAGTGGTTCAT TCCGGTAGACCTATCGGTTG	8	0.22	264–297

All annealing temperatures were 57°C.

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