

The evolution of soldier reproduction in social thrips

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We estimated the degree of reproductive differentiation between foundresses and soldiers in multiple populations of five species of haplodiploid Australian gall-forming thrips using microsatellite data, ovarian dissections, and census data. Microsatellite-based species estimates of average per capita reproductive output of soldiers relative to the foundresses ranged from 0.005 to 0.64, and dissection and census-based estimates ranged from 0.17 to 1.1. Mapping of these estimates onto a phylogeny showed that levels of soldier reproduction were apparently higher in three basal lineages than in two more derived lineages. We infer from this phylogenetic pattern that soldier morphology and behavior of thrips evolved in the presence of substantial levels of soldier reproduction. This pattern of evolutionary change is similar to that proposed for the origin of soldiers in aphids and termites, but it differs from the scenario proposed for the origin of workers in Hymenoptera, within which helping and strong reproductive division of labor apparently evolved before morphological differentiation. We suggest that this difference in evolutionary routes to eusociality between taxa with soldiers and taxa with foraging workers was driven by a weaker trade-off between helping and reproducing, and a greater ability of the helpers to withstand reproductive domination, in taxa with soldiers. This is the first study to analyze the social-evolutionary trajectories of reproductive, behavioral, and morphological differentiation in the context of a species-level phylogeny. **Key words:** castes, inbreeding, microsatellites, soldiers, thrips. [*Behav Ecol* 13:519–525 (2002)]

Reproduction by helpers is a common feature of social insects that is closely linked to the evolution of many colony characteristics, including dominance behavior, egg cannibalism, brood destruction, matricide, reduced efficiency, and rarity of physical castes among helpers (Bourke, 1988; Strassmann, 1988; Woyciechowski, 1989). Indeed, Trivers and Hare (1976) suggested that production of sons by incipient helpers facilitated the origin of eusociality in haplodiploids, and several theoretical studies have substantiated a potential role for parthenogenic male production to increase the ease of helper evolution (Aoki and Moody, 1981; Bartz, 1982; Crozier and Pamilo, 1996; Iwasa, 1981; Pamilo, 1984, 1991). Despite the importance of helper reproduction for the origin and evolution of eusociality, its prevalence and coevolution with other social traits have yet to be analyzed in an explicitly phylogenetic context.

We believe that analysis of soldier reproduction in the social, haplodiploid gall-forming thrips of Australia holds particular promise for understanding the origin and evolution of helper reproduction because (1) soldier mating and egg laying appear to be prominent features of social thrips (Crespi, 1992a; Kranz et al., 1999, 2001a,b) and (2) a well-supported phylogeny for the gall-forming thrips of Australia exists (Crespi et al., 1998; Morris, 2000), allowing inference of the evolutionary history of soldier reproduction. In this study we estimated the extent of soldier reproduction in multiple popu-

lations of five species of gall-forming thrips in Australia. We estimated levels of soldier reproduction using two approaches. First, we used microsatellite genetic markers and a population genetic model described below. Second, we used ovarian dissection and colony census data. Here we evaluate the resulting multispecies pattern of soldier reproduction, in a phylogenetic context, and we discuss the role that soldier reproduction may have played in the origin and evolution of eusociality in thrips and other insects.

METHODS

Study populations and natural history

The gall-forming thrips of Australia are composed of three genera: *Onychothrips*, *Kladothrips*, and *Oncothrips*, with eusocial species occurring in the latter two genera (Crespi et al., 1998). Gall-forming thrips are mostly restricted to the arid and semiarid zones of Australia, and each thrips species is associated with specific *Acacia* host tree species (Crespi and Mound, 1997). A colony is initiated by a single female (or, in some species, a single female with a male) who induces gall formation on a growing phyllode of a host *Acacia* tree. Enclosed within the gall, the founding female lays her eggs. The first individuals of a foundress's brood to eclose are the gall-bound soldiers, which are morphologically and behaviorally specialized for defense, with reduced wings and enlarged forelegs (Crespi, 1992a). The soldiers include both sexes (Crespi, 1992a,b; Crespi and Mound, 1997; Crespi et al., 1997). The foundress's life span overlaps with the soldiers, but she dies before the dispersing brood reaches the adult stage. Studies show that female soldiers are often inseminated provided there were male soldiers in the gall (Kranz et al., 2001b). Demographic collection data indicates that if soldiers reproduce,

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Table 1
Colony demographics for each collection, and estimates of average reproductive skew from microsatellite data (M; %*spfd*: percentage of total female dispersers produced by soldiers) and census data (C; %*spfd*: percentage of total dispersers produced by soldiers)

Collection (# of galls)	Female soldiers/gall	% Female soldiers	# Dispersers/ gall	% Female dispersers	$F_{IS(D)}$
<i>hamiltoni</i> 1 (10)	5.9 ± 2.9	48.0 ± 21.0			0.53 ± 0.188
<i>hamiltoni</i> 2 (19)	4 ± 2.6	33.7 ± 20.4			0.82 ± 0.035
<i>hamiltoni</i> 3 (11)	6.5 ± 3.3	47.6 ± 7.8			0.74 ± 0.056
<i>hamiltoni</i> (census) (35)	14.0 ± 8.4	49.2 ± 0.03	140.0 ± 63	94.4 ± 0.01	
<i>morrissi</i> 1(20)	9.0 ± 5.2	72.7 ± 10.1			0.40 ± 0.087
<i>morrissi</i> 2(7)	13.5 ± 2.9	68.4 ± 6.6			0.51 ± 0.032
<i>morrissi</i> (census) (35)	24.0 ± 8.7	67.0 ± 0.31	744.0 ± 170	69.0 ± 0.09	
<i>waterhousei</i> 1 (7)	9.33 ± 3.9	84.0 ± 13.7			0.02 ± 0.09
<i>waterhousei</i> (census) (41)	10.0 ± 3.0	92.0 ± 0.02	105.0 ± 26	49.0 ± 0.28	
<i>tepperi</i> 1 (8)	3.8 ± 0.8	89.8 ± 15.5	101.5 ± 20.0	92.8 ± 3.0	0.39 ± 0.049
<i>tepperi</i> 2 (12)	3.25 ± 1.53	100.0 ± 0.0	68.8 ± 17.2	89.9 ± 5.7	0.28 ± 0.051
<i>tepperi</i> (census) (24)	8 ± 0.12	77.0 ± 4.08	57 ± 1.74	85.0 ± 4.90	
<i>habrus</i> 1 (15)	11.9 ± 4.9	75.9 ± 17.3			0.30 ± 0.025
<i>habrus</i> 2 (7)	8.7 ± 5.0	97.1 ± 7.6			0.32 ± 0.150
<i>habrus</i> (census) (24)	6 ± 3.0	88.0 ± 0.53	140 ± 63	49.0 ± 0.26	

The soldier inbreeding estimates for all collections, except *waterhousei* 1, appear elsewhere (Chapman and Crespi, 1998; Chapman et al., 2000). Standard deviations are reported in all cases. Standard deviations for %*spfd* were calculated by jackknifing, a general-purpose statistical technique useful when the distributional assumptions are not clear (Sokal and Rohlf, 1981). An absolute difference between $F_{IS(D)}$ and the negative $F_{IS(S)}$ in *waterhousei* 1 was used to calculate %*spfd*. The reproductive output of an individual soldier relative to the foundress production of dispersers was calculated in three ways and listed in the last three columns (see footnotes a, b, and c below).

^a Estimates = %*spfd*/(female soldiers/gall)/(no. of galls/%*spfd*).
^b For this calculation, we assume that all disperser males are produced by the soldiers and that the sex ratio of dispersers determined in the census population is representative for a species. Estimates = [%*spfd* (% female dispersers + (1-% female dispersers))]/[(female soldiers/gall)/(1-%*spfd* (% female dispersers))].
^c Estimates are calculated as average soldier chorionated oocyte count divided by the average foundress chorionated oocyte count.
^d No standard deviation for this estimate because it was derived indirectly (see Methods).

they could only be producing dispersers (Crespi, 1992a,b, unpublished data).

In this article, we use the term “helper” to refer to both workers and soldiers. As defined here, workers are individuals specialized for foraging, brood rearing, or both, and soldiers are morphologically and behaviorally specialized to defend their colony. A group of soldiers represents a caste, a defining characteristic of eusociality, only if there is a reproductive division of labor (Crespi and Yanega, 1995).

Collection data for the five gall-forming species with soldiers in our study are listed in Appendix A. At each collection site, galls were taken from a stand of several trees in close proximity, such that population subdivision above the level of the individual gall is unlikely to contribute to any deviations from Hardy-Weinberg expectations (Blows and Schwarz, 1991; Pamilo, 1985). Collections used in genetic analysis for four species, *Kladothrips hamiltoni*, *Oncothrips habrus*, *Oncothrips morrissi*, and *Oncothrips waterhousei* contained a foundress female, adult soldiers, first- and second-instar larvae, and some eggs. The collections of *Oncothrips tepperi* used in genetic analysis contain soldiers and adult dispersing brood.

Microsatellite markers

Genetic data were gathered using microsatellite markers developed specifically for gall-forming thrips. Data collections were made for foundresses and soldiers for four species: *K. hamiltoni*, *O. habrus*, *O. waterhousei*, and *O. morrissi*. For *O. tepperi* genotype data were collected for all soldiers in the two

populations sampled, and a 25% random sample of the adult female dispersing brood (males are hemizygous and therefore are not useful for estimating homozygosity levels; see model below) from each gall in our collections was also genotyped. The preparation of samples, the determination of microsatellite genotypes, and primer sequences used in this study are reported in Chapman et al. (2000). A summary of loci used and number of alleles found in each case is presented in the Appendix.

Model for estimating soldier production of female dispersers

Dispersing brood can be produced in the gall of a thrips species with soldiers in two ways: (1) the foundress produces soldiers and dispersers, and (2) the foundress produces soldiers and the soldiers produce all or some of the dispersers. If the foundress produces all of the female dispersers, then the inbreeding coefficient (F_{IS}) is expected to be identical for the soldiers and the dispersers (i.e., F_{IS} measured using soldier genotypes is equal to F_{IS} measured using disperser genotypes). If, however, soldiers produce all the dispersing females (via sib-mating), then the inbreeding estimate using disperser genotypes is expected to increase over that of the soldiers, and the level of increase expected can be determined using the inbreeding recursion equation for full-sib mating: $F_{IS(\text{generation } t+1)} = \frac{1}{4} + \frac{1}{2}F_{IS(t)} + \frac{1}{4}F_{IS(t-1)}$ (Laidlaw and Page, 1986). When soldiers produce only a proportion of dispersing females, the inbreeding coefficient measured in the dispersing generation (female dispers-

Table 1, extended

$F_{IS(S)}$	% <i>spfd</i> (M)	% <i>spd</i> (C)	Per capita production by soldiers relative to foundress		
			Female soldiers/gall (M) ^a	Female soldiers and dispersers/gall (M) ^b	Total (C) ^c
0.44 ± 0.088	55.3 ± 22.8	86.0 ± 0.03	0.21 ± 0.09	0.23	0.47
0.77 ± 0.023	72.0 ± 7.2		0.64 ± 0.06	0.70	
0.69 ± 0.027	53.9 ± 22.5		0.18 ± 0.08	0.20	
0.28 ± 0.045	57.0 ± 20.4	96.0 ± 0.04	0.15 ± 0.05	0.26	1.1
0.33 ± 0.036	85.0 ± 9.0		0.42 ± 0.04	0.64	
−0.13 ± 0.06	52.2 ± 5.9	83.0 ± 0.05	0.12 ± 0.01	0.35	0.47
0.35 ± 0.049	23.2 ± 3.9	40.0 ^d	0.08 ± 0.01	0.11	0.07
0.27 ± 0.052	5.0 ± 8.4		0.016 ± 0.03	0.052	
0.31 ± 0.038	5.6 ± 10.7	54.0 ± 0.05	0.005 ± 0.01	0.10	0.15
0.28 ± 0.120	30.5 ± 23.8		0.05 ± 0.04	0.22	

ers produced by the foundress or by soldiers) increases proportionately to the extent that soldiers produce female dispersers. Thus, the inbreeding coefficient of the current generation of dispersing females [$F_{IS(D)}$] can be estimated using the inbreeding coefficient of the soldiers [$F_{IS(S)}$], the previous generation of dispersers [$F_{IS(D-1)}$], soldier-produced female dispersers (*spfd*), and the inbreeding recursion equation for full-sib mating [$1/4 + 1/2 F_{IS(S)} + 1/4 F_{IS(D-1)}$] in the following equation:

$$F_{IS(D)} = F_{IS(S)}(1 - \text{spfd}) + \text{spfd}[1/4 + 1/2F_{IS(S)} + 1/4F_{IS(D-1)}]. \quad (1)$$

When soldiers produce none of the female dispersers (*spfd* = 0), the above equation reduces to $F_{IS(D)} = F_{IS(S)}$ as expected. When soldiers produce all the female dispersers (*spfd* = 1), the inbreeding coefficient of the dispersers increases by the increment predicted by the recursion equation for one generation of full-sib mating [$F_{IS(D)} = 1/4 + 1/2F_{IS(S)} + 1/4 F_{IS(D-1)}$].

Equation 1 assumes that soldier females mate only with siblings. This assumption is reasonable given that galls are initiated by a single female and gall membership appears to be stable (Chapman and Crespi, 1998).

Genetic data from two generations of gall initiation would be difficult to obtain for the gall-forming thrips. Equation 1 can be simplified for a single-season collection of galls by assuming $F_{IS(D)}$ is at equilibrium such that $F_{IS(D)} = F_{IS(D-1)}$ and solving Equation 1 for *spfd*:

$$\text{spfd} = [F_{IS(D)} - F_{IS(S)}]/[1/4 - 1/2F_{IS(S)} + 1/4F_{IS(D)}]. \quad (2)$$

Simulations indicate that if the assumption of inbreeding equilibrium is not met, estimates of *spfd* using Equation 2 will still tend to accurately approximate the true value (Chapman, 2000).

Ovarian dissection and census data collections for estimating soldier production of female and male dispersers

For the collections *K. hamiltoni* (census), *O. morrisi* (census), *O. waterhousei* (census), and *O. habrus* (census), foundresses

and a sample of soldiers from each gall were dissected to determine the number of chorionated oocytes in each morph (see Kranz et al., 1999). Oocytes that are chorinated have hexagonal reticulation that is characteristic of all eggs in the tubuliferan suborder and can be viewed with a light microscope. The number of soldiers dissected varies between species analyzed, but ranges from 71 to 224. For these estimates we assume that fecundities of foundresses and soldiers are positively correlated with the number of chorionated oocytes. Chorionated oocyte counts therefore represent the relative contribution of the foundress and soldiers to the dispersing brood. We dissected both morphs when they were likely producing eggs destined to be dispersers (i.e., when gall brood size was larger than the maximum number of soldiers expected for a species). For *O. tepperi* (census), regressions of the number of dispersers on the number of female soldiers were conducted to estimate the proportion of the disperser brood that was produced by the foundress (the y intercept). The number and sex ratio of soldiers and dispersers were ascertained from multiple gall collections throughout the life cycle of each species.

Statistical analysis

Inbreeding calculations using microsatellite data were conducted using the computer program Relatedness 4.2c (Goodnight KF, Rice University). Formulae used in this computer program are described in Queller and Goodnight (1989).

RESULTS

Microsatellite estimates of the proportion of female dispersers produced by soldiers

Inbreeding coefficients in soldiers and dispersers were estimated for all five species (Table 1). Inbreeding estimates ranged from 0.0 to 0.8, with the highest values found for *K. hamiltoni*. Inbreeding values for dispersers were higher than those of soldiers in all populations, and estimates of the proportion of soldier-produced female dispersers (*spfd*) ranged from 5% to 85% (Table 1).

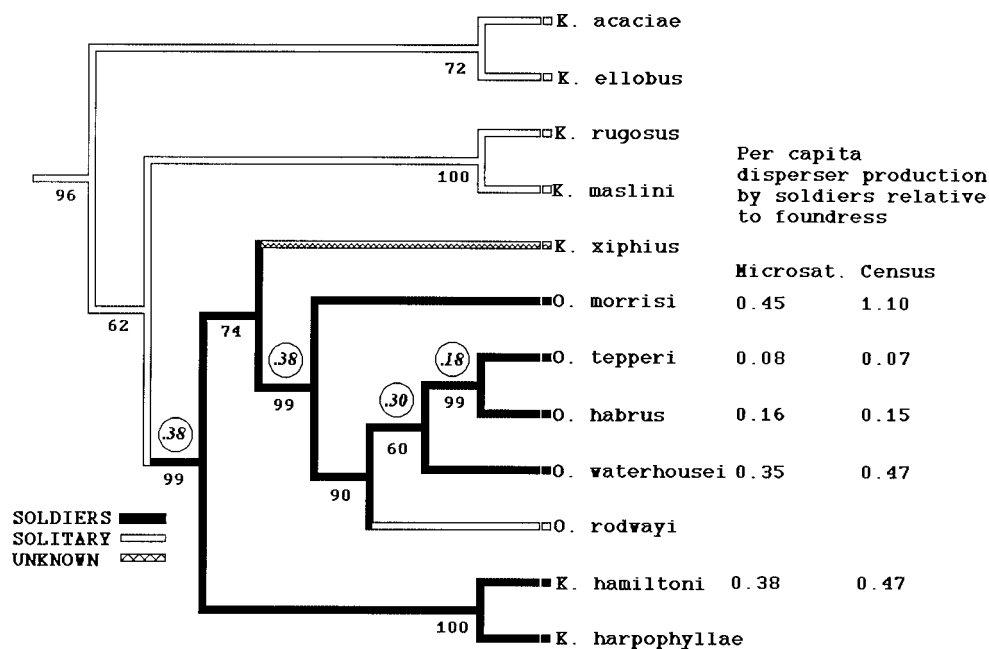


Figure 1
Data from the genes EF-1 α , 16S stems, cytochrome oxidase I, and wingless were analyzed together to infer a phylogeny of solitary and eusocial thrips on *Acacia* host trees within the section Plurinerves (Morris, 2000). Analysis using maximum-parsimony and maximum likelihood produced the same topology. Maximum likelihood bootstrap values (100 replicates) are below branches, and they are notably high for all of the nodes supporting the derived status of (*O. tepperi* + *O. habrus*) relative to the other species with soldiers. Estimates of per capita soldier production of male and female dispersers using microsatellite data (Table 1) were averaged for each species and mapped onto the phylogeny along with census per capita estimates of soldier production of dispersers. Circled numbers at nodes are ancestral levels of per capita disperser production by soldiers inferred using squared change parsimony and microsatellite data under the assumption that soldiers produce the disperser males. We do not yet know if *Kladothrips xiphius* has soldiers, and the single origin of soldiers represented in the figure does not consider *K. xiphius* in the analysis.

Soldier per capita production of female dispersers (*spfd*/number of female soldiers) relative to the foundress contribution ($1 - spfd$) ranged from 0.005 to 0.64 (Table 1). Per capita estimates were also calculated under the assumption that the soldiers produce all of the dispersing males, and these per capita estimates ranged from 0.052 to 0.70 (Table 1).

Ovarian dissection and census data estimates of soldier production of female and male dispersers

The proportion of dispersing males and females produced by soldiers was estimated for all five species and ranged from 40% to 96% (Table 1). Estimates of soldier reproduction based on these data were consistently higher than estimates, for the same species, using microsatellite data (Table 1). The broad pattern seen in the genetic data of relatively high (*O. waterhousei*, *O. morrisi*, and *K. hamiltoni*) and low (*O. habrus* and *O. tepperi*) soldier reproduction is also apparent from these estimates.

Soldier per capita production of dispersers relative to the foundress contribution ranged from 0.07 to 1.1 (Table 1). These estimates were generally higher than per capita estimates using microsatellite data for a given species.

Production of male dispersers by soldiers in the population *O. tepperi* 2 (the only species for which we had both completely developed broods and microsatellite data) is supported by a regression of number of male dispersers on number of female soldiers present in the gall ($y = 1.45 + 2.26x$, $r^2 = .61$, $p = .0002$). Soldiers in this population are each producing approximately 4–5 (4.7) disperser males (6.95 disperser males per colony minus the foundress's contribution, the y intercept). Male production by soldiers in *O. tepperi* 2 is consistent

with no male soldiers being present in this population to act as a source of sperm. In population *O. tepperi* 1, where soldier males were present, there was no association between number of female soldiers with the number of male dispersers present in the gall ($r^2 = .03$, $p = .62$). Therefore, we have no evidence for soldier production of males in this population.

Concordance of estimates from microsatellite data and data from ovarian dissections and census

We analyzed concordance of the microsatellite data with the ovarian dissection and census data via the correlations between the soldier reproduction estimates from the two methods. Microsatellite per capita estimates (of female production) were positively correlated with ovarian dissection and census estimates (of male and female production; $r = .722$), but this relationship was not significant ($p = .168$, $n = 5$). However, there was a strong and significant correlation between per capita dissection and census estimates, and the per capita microsatellite estimates, when soldiers are assumed to produce all of the male dispersers ($r = .891$, $p = .0425$, $n = 5$).

Inference of ancestral states of reproductive differentiation

To understand the evolution of soldier reproduction, we mapped census and microsatellite estimates onto a phylogeny of Australian gall-inducing thrips (Figure 1). The three relatively basal species with soldiers (*K. hamiltoni*, *O. morrisi*, and *O. waterhousei*) had substantially higher soldier reproduction than the derived species *O. tepperi* and *O. habrus*. Character optimization using squared-change parsimony in MacClade (Maddison and Maddison, 1992) shows that levels of inferred

APPENDIX

Collection data and microsatellite primers used for each species

Species collection	Collection site and date	No. of galls	Microsatellite primers	No. of alleles
<i>Kladothrips hamiltoni</i> 1	81.2 km east of Cadney Park, South Australia, 24 April 1997	10	HamGATA1	4
<i>Kladothrips hamiltoni</i> 2	41 km south of Oodnadatta, South Australia, 28 February 1996	19	HamGATA1	5
<i>Kladothrips hamiltoni</i> 3	47 km east of Birdsville, Queensland, 7 April 1998	11	HamGATA1	5
<i>Kladothrips hamiltoni</i> (census)	Two sites: Arckaringa Creek Homestead, South Australia (28 December 1995; 6, 15, 30 January, 30 March, 30 April, 14 May 1996) and 41 km southeast of Arckaringa Creek (40 km south of Oodnadatta) Homestead (31 May 1997; 25 February 1998; 7 January, 9 March 1999)	35		
<i>Oncothrips morrissi</i> 1	139 km west of Oodnadatta, South Australia, 29 February 1996	20	MorAAT1	8
<i>Oncothrips morrissi</i> 2	20 km east of Curtin Springs, Northern Territory, 7 March 1996	7	MorAAT1	11
<i>Oncothrips morrissi</i> (census)	Four sites: Curtin Springs, Northern Territory, 7 May 1996; 50 km and 84 km north of Coober Pedy, South Australia, (6 January 1999, 3 February 1999, 9 March 1999, 21 April 1999) 4 km north of the South Australia and Northern Territory border between Marla and Kulgera, 6 January 1999	73		
<i>Oncothrips waterhousei</i> 1	Middleback, South Australia, 20 March 1996	7	TepAAT2	9
<i>Oncothrips waterhousei</i> (census)	Three sites: Middleback, South Australia, 4 May 1995–21 March 1999, 38 km south of Port Augusta, South Australia, 26 January 1996, 30 May 1998, 9 March 1999, 21 km from Glendambo toward Tarcoola, 16 March 1996	41		
<i>Oncothrips tepperi</i> 1	35 km south of Gilgandra, New South Wales, 14 June 1993	8	TepAAT1 TepAAT2	5 3
<i>Oncothrips tepperi</i> 2	1.5 km west of Mathong, New South Wales, 23 June 1993	12	TepAAT1 TepAAT2	5 4
<i>Oncothrips tepperi</i> (census)	Middleback, South Australia, 17 February, 12 March, 8 April, 23 April, 22 May 1997	24		
<i>Oncothrips habrus</i> 1	46 km southeast of Warren, New South Wales, 14 June 1993	15	TepAAT1 TepAAT2	10 8
<i>Oncothrips habrus</i> 2	2.3 km south of Ivanhoe, New South Wales, 20 June 1996	7	TepAAT1 TepAAT2	8 6
<i>Oncothrips habrus</i> (census)	Two sites: 46 km east of Warren, New South Wales, 25 September 1996, 21 km southwest of Mildura, Victoria, 26 February, 3 April, 22 May, 31 July 1999	24		

Collections from which only census data were collected are indicated by the word “census” in brackets after the species name. Microsatellite loci primer sequences were published in Chapman and Crespi (1998) and Chapman et al. (2000).

ancestral reproductive differentiation were relatively low at more-basal nodes (Figure 1).

DISCUSSION

This is the first evidence, from both genetic and phenotypic data, conclusively demonstrating soldier reproduction in thrips and putting soldier reproduction in an explicitly phylogenetic context. Analysis of ovarian dissection and census data, and microsatellite data, indicates that average levels of

reproductive differentiation between foundresses and soldiers vary substantially between species. Reproductive differentiation is relatively low in *K. hamiltoni*, *O. waterhousei*, and *O. morrissi*, but there was substantially higher differentiation in *O. tepperi* and *O. habrus*. Indeed, microsatellite data analysis for two *O. habrus* and two *O. tepperi* populations indicate that some soldiers in these species may approach functional sterility.

In our study, estimates of soldier reproduction using chorionated oocyte counts and regression analysis are higher than

estimates made using genetic data. One difference between methods is that genetic estimates are based on female disperser production only, whereas census estimates include production of both males and females. If soldiers produce more males than the foundress does, then genetic data will underestimate egg production by soldiers. We observe that where sex ratios are highly female biased (i.e., in *K. hamiltoni* and *O. tepperi*), per capita production by soldiers relative to foundress falls within or close to the range of microsatellite-based estimates. Moreover, per capita microsatellite-based estimates under the assumption that soldiers produce all of the male dispersers exhibit a higher correlation with per capita census estimates than do the microsatellite-based estimates without this assumption.

A second difference between methods is that census data estimates assume that all chorionated oocytes will result in viable offspring, while microsatellite estimates use only genotypes of adult thrips. If this assumption is violated, as a result of egg retention or egg resorption by soldiers, then census data provide overestimates of soldier reproduction. We conclude that the genetic estimates are more likely to be underestimates of the true level of soldier reproduction, and the census data are more likely to provide overestimates. However, these differences do not affect our conclusions that soldiers often produce a substantial proportion of dispersers within a colony and that reproductive differentiation differs markedly between species.

From our phylogenetic pattern of lower levels of reproductive differentiation in more basal lineages (Figure 1), we infer that soldier morphology and behavior evolved in the context of substantial levels of reproduction by soldiers and that reduced reproduction by thrips soldiers has evolved more recently. Factors directing this change to reduced soldier reproduction are unknown. However, we note that an association may exist between the evolution of decreased gall size (Crespi and Worobey, 1998) and a decrease in soldier reproduction. Soldier reproduction in small galls may be limited by space, and female soldiers may not be inclined to replace highly related sisters with their own less related daughters (Kranz et al., 1999; Ratnieks, 1988).

Our evidence for thrips indicates that morphological differentiation evolved before strong reproductive division of labor. Might similar patterns exist for other taxa with soldiers? In aphids, willingness to defend a clone apparently evolved first, followed by morphological specialization for defense, with soldiers initially capable of molting to adulthood (Stern and Foster, 1997). Time spent as a soldier instar may have increased until there was selection for a split in developmental pathways that allowed a more rapid development of nonsoldiers. Ultimately, in some species, soldiers delayed molting until they became obligately sterile. Termite soldiers may also have evolved under high levels of reproduction in *Archotermopsis*, which are regarded as the most socially primitive termites (Thorne, 1997), all soldiers have well-developed gonads, and similar apparent high levels of soldier reproduction are known from other species in the primitive family Termopsidae (Myles, 1986).

Thrips, termites, and aphids may thus share a similar scenario for the origin of the helpers, which involved the emergence of helping behavior and morphological specialization in association with the retention of substantial reproduction, followed by a decrease in reproduction in some lineages. In contrast, the evolution of helping in Hymenoptera involved the origin of workers being directly and immediately associated with marked reproductive division of labor, and both preceded morphological differentiation (Bourke and Franks, 1995; Wilson, 1971). We suggest that acting as a soldier during rare, episodic colony invasions involves less of a trade-off be-

tween helping and reproduction than does acting as a Hymenopteran worker and engaging in the daily activities of foraging or brood care. The energetic demands of worker tasks may direct energy away from reproduction, while there are no increased energy demands on soldiers when a colony is not threatened except in the case of gall cleaning, or patrolling the gall surface, in some species of social aphids (Benton and Foster, 1992; Stern and Foster, 1996). We also note that soldier morphology may make physical and reproductive domination of helpers by foundresses more difficult than in taxa without such morphological specializations for fighting. The differences between taxa with soldiers and taxa with workers may ultimately derive from aspects of ecology, as taxa with soldiers inhabit defensible factory fortresses, while in many taxa with workers, helping behavior apparently evolved in the context of high forager mortality and "life insurance" for the helpless brood (Crespi, 1994; Queller and Strassmann, 1998).

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