

Inbreeding Ancestors: The Role of Sibmating in the Social Evolution of Gall Thrips

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Abstract

We used microsatellite data to estimate levels of inbreeding in four species of solitary gall thrips that are in the same clade as the six species with soldier castes. Three of the four species were highly inbred (F_{is} 0.54–0.68), and the other apparently mated randomly (F_{is} near zero). These estimates, combined with previous data from species with soldiers, suggest that inbreeding is a pervasive life-history feature of the gall-inducing thrips on Australian *Acacia*. Mapping of inbreeding estimates onto the phylogeny of the gall inducers showed that the ancestral lineage that gave rise to soldiers was apparently highly inbred, and therefore, inbreeding could have played a role in the origin of sociality within this group. Moreover, there was a trend from high levels of inbreeding at the origin of soldiers to low levels in the most derived species with soldiers, which exhibits the highest levels of reproductive division of labor and soldier altruism. These patterns are consistent with considerations from population genetics, which show that the likelihood of the origin of soldier altruism is higher in inbreeding populations but that, once soldiers have evolved, a reduction in inbreeding levels may facilitate the evolution of enhanced division of labor and reproductive skew.

Mating between relatives has three important effects on social evolution. First, such inbreeding has been shown theoretically to increase the likelihood of genes for social behavior spreading to fixation within a population (Breden and Wade 1981, 1991; Craig 1982; Hamilton 1972; Michod 1980, 1993; Price 1970; Uyenoyama 1984; Wade and Breden 1981, 1987). In particular, inbreeding leads to higher among-group genetic variation, elevated within-group relatedness, and lower within-group variance in relatedness (Shields 1982), which can facilitate the evolution of altruism by kin selection. Especially high relatedness due to inbreeding has been documented in naked mole rats (Reeve et al. 1990), social thrips (Chapman et al. 2000), and social spiders (Avilés 1997). However, its role in the evolution of sociality has yet to be documented empirically because among-population variation in inbreeding or variation in time along a phylogeny has not been linked to spatial or temporal variation in social traits.

Second, in addition to elevating relatedness, inbreeding can also affect social behavior through its influences on relatedness asymmetries from potential breeders to collateral relatives versus offspring. For example, inbreeding can reduce the relatedness asymmetries engendered by haplodiploidy (Hamilton 1972), or if outbred colony founders

produce offspring that inbreed in a multigenerational colony, relatedness asymmetries could be created (in diploids, Bartz 1979; Myles and Nutting 1988; Tyson 1984) or enhanced (in haplodiploids, Chapman 2003).

Finally, the avoidance of mating between relatives, due to the fitness-reducing effects of inbreeding depression, can have profound effects on the presence and form of social interactions (e.g., Cockburn 1998; Solomon and French 1997). For example, incest avoidance in family groups subject to strong ecological constraint can favor natal philopatry and helping behavior by offspring (Bennett et al. 1999). Alternatively, where ecological constraints are weaker, inbreeding depression can select for dispersal by one or both sexes of offspring before sexual maturity, which prevents social groups from forming at all (e.g., Gandon 2000). Only if strong inbreeding persists for sufficient generations to purge diploid genomes of deleterious recessives can the costs of inbreeding depression be mitigated or overcome.

Deleterious consequences of inbreeding are expected and common in diploid taxa, but in haplodiploids the situation is more complex. Under this genetic system, detrimental alleles are exposed to selection in the haploid males and removed rapidly (Crozier 1977; Hedrick and Parker 1997), such that

Table 1. Demographic and genetic data for gall-inducing thrips on Australian *Acacia* with soldiers

Species	No. soldiers	No. dispersers	Soldier % male	Disperser % male	Foundress inbreeding level F_{is}	Soldier inbreeding level F_{is}	Soldier reproduction, msat
<i>Kladothrips habrus</i> (from <i>Acacia melvillei</i>)	10	105	16	51	0.31	0.30	0.16
<i>Kladothrips hamiltoni</i>	25	115	52	6	0.70	0.63	0.38
<i>Kladothrips intermedius</i>	15	60	21	15	0.34	0.31	0.07
<i>Kladothrips morrisi</i>	38	619	25	41	0.45	0.31	0.45
<i>Kladothrips waterhousei</i> (from <i>Acacia papyrocarpa</i>)	7	79	9	47	0.02	-0.13	0.35

Data are from (Chapman et al. 2000, 2002). "Soldier reproduction, msat" refers to the per capita reproduction of female soldiers, relative to that of foundresses.

they are seldom expressed in the homozygous form in females. However, in haplodiploid taxa with chromosomal, single-locus sex determination, inbreeding can lead to the production of diploid males, which are sterile and reduce colony fitness (Cook 1993). Diploid male production could thus favor incest avoidance, dispersal, or helping in Hymenoptera or other haplodiploids.

Eusociality, in the form of soldier castes, has been described in six species of haplodiploid gall-inducing thrips on Australian *Acacia* (Crespi and Mound 1997; Crespi et al. 1997; Morris et al. 2002). These species are especially useful for analyzing the role of inbreeding in social evolution because population-genetic (microsatellite) data have shown that inbreeding is common in foundresses and soldiers of the eusocial species, yet its strength varies substantially among the species (Table 1). Moreover, a robust, species-level phylogeny is available comprising the social species and their non-social relatives (Morris et al. 2001, 2002), which allows inference of relatedness and inbreeding levels for the branch subtending the inferred single origin of the soldier caste (Chapman et al. 2000). Inbreeding and relatedness estimates mapped onto the phylogeny of the gall inducers have led to the inference of unusually high relatedness due in part to high levels of inbreeding, near to the origin of soldiers, which suggest a role for inbreeding in the evolution of helping behavior within this clade (Chapman et al. 2000). However, because only species with soldiers have been studied thus far, it is difficult to ascertain if inbreeding by foundresses evolved before, coincident with, or soon after the origin of soldiers.

In this paper, we present data on levels of inbreeding in solitary relatives of social thrips, which allow phylogeny-based testing among alternative hypotheses for the role of inbreeding in the origin of soldiers. By the first hypothesis, the presence of inbreeding in solitary species would suggest that inbreeding evolved prior to the origin of soldiers. Alternatively, if inbreeding were absent in solitary species related to social ones, then it presumably evolved along the same branch of the phylogeny on which soldiers originated. Differentiating among these hypotheses is critical to elucidating the population-genetic structure under which soldiers evolved in gall thrips, with implications for the importance of inbreeding in social evolution among all animals.

Methods

Natural History of Gall-Inducing Thrips on Australian *Acacia*

Formerly, the gall-inducing thrips comprised three genera: *Kladothrips* Froggatt, *Kladothrips* Karny, and *Kladothrips* Karny. The last two genera have now been synonymized with the genus *Kladothrips* (Crespi et al. 2004). This generic synonymy has required the reversion of one species name, *Kladothrips tepperi*, to its junior synonym, *intermedius*, so that the name becomes *Kladothrips intermedius* Bagnall (Crespi et al. 2004). Thrips induce galls on young, actively growing phyllodes (modified petioles that function as leaves) of *Acacia* (Crespi 1992a,b; Crespi et al. 1997; Mound 1994; Mound et al. 1996). The gall has been described as a "factory fortress" because it provides both food and shelter for all occupants and is therefore essential for their survival and reproduction (Chapman et al. 2002; Crespi 1994; Queller and Strassmann 1998). A single foundress produces a single gall in which she becomes interred. In species with soldiers, the first of the foundress's brood to eclose become gall-bound soldiers. Soldiers, which include both sexes in varying proportion among species (Table 1), typically have short or no wings, enlarged forelegs, and reduced antennae compared to foundresses and winged (disperser) males. Soldiers use their enlarged forelegs to defend the gall against kleptoparasitic thrips species in the genus *Koptothrips* Bagnall, which invade galls, kill the occupants, and then produce a brood of their own. *Koptothrips* are thought to be the major selective force underlying soldier behavior and morphology, specifically the enlarged, armed forelegs (Crespi 1992a,b, 1996; Crespi and Mound 1997; Perry et al. 2003; Simpkins 2002).

Soldiers develop in a distinct cohort, at least several weeks before the eclosion (molting to adulthood) of the fully winged dispersers. Dispersers of both sexes eclose near the end of the gall life cycle or shortly after leaving the gall, and they constitute the next generation of gall inducers. This dispersing generation is derived not just from foundress-produced eggs but also from a single generation of soldier incest that yields additional dispersers. Thus, soldiers produce dispersers but not additional soldier offspring. Diploid male production has not been detected in these thrips or other thrips

species (Chapman and Crespi 1998; Crespi 1991), which suggests that Thysanoptera do not have chromosomal sex determination.

The fecundity of female soldiers differs substantially among thrips species (Chapman et al. 2002). The reproductive output by soldiers varies from comparable levels to that of the foundress (in *Kladothrips morrisi* Mound, Crespi and Kranz, Kranz et al. 2001) to only a few parthenogenetically produced male offspring (in some populations of *K. intermedius*, Chapman et al. 2002). Mapping of per capita offspring production by soldiers (relative to foundresses) onto the phylogeny of the gall inducers led to the inference that levels of reproduction by soldiers were highest near to the origin of this morph (Chapman et al. 2002). Subsequent evolution in this lineage resulted in increased behavioral and morphological specialization of soldiers for defense and a strong reproductive division of labor with near-zero reproduction by soldiers in two species. Correlative evidence is consistent with the hypothesis that such decreased soldier fecundity was due at least in part to a trade-off between reproductive output and defensive ability (Perry et al. 2003; Simpkins 2002).

Collections

Four species of solitary thrips were collected from the Australian arid zone from one or two locations, and one species was collected from the same site in two different years (Table 2). All collections were made from clusters of adjacent trees such that populations are highly unlikely to exhibit genetic substructure (Pamilo 1985) above the level of the individual gall—the distances a disperser can traverse have been shown to be in excess of 1 km for at least one species, *K. intermedius* (McLeish et al. 2003). In *Kladothrips xiphius* Mound Crespi and Kranz and *Kladothrips rugosus* Froggatt, the founding adults (the female, and in some galls, a male as well) were the only adult thrips enclosed within the gall and they were easily identified. *Kladothrips rugosus* on the host *Acacia papyracarpa* exhibits a polymorphism in gall morphology (Table 1), and individuals were labeled as to the morphology of their gall. The two forms of *K. rugosus* were collected from the same cluster of adjacent trees at one location. We suspected that they were different species, grouped them as sister taxa based on their substantial morphological similarities, analyzed them separately, and presented separate estimates. In *Kladothrips antennatus* (Moulton) comb. n. and *Kladothrips arotrum* (Mound) comb. n., the founding female was not easily discernible from the brood because they had eclosed. In these cases, 10 adult females per gall were sampled and grouped as gall members for genetic analysis. Haplod male thrips are useful in genetic analysis for identifying null alleles, cases where a change in a primer adherence site leads to a nonamplification (Jarné and Largoda 1996). Females were identified by observing their body size and the shape of the ninth abdominal segment (Crespi 1993). These external evaluations of gender were confirmed for a subset of each species by viewing the fustis of females and phallobase of males in semicleared abdomens (abdomens were mounted on microscope slides in Hoyer's

medium and heated overnight). In all cases, abdomens of adult females were removed to avoid contamination in genetic analysis by the sperm stored in inseminated females' spermathecae.

Microsatellite Analysis

Microsatellites are codominant genetic markers exhibiting high levels of length polymorphisms inherited in a simple Mendelian fashion (Ashley and Dow 1994; Queller et al. 1993). To generate our microsatellite data, we isolated and characterized 24 microsatellite loci from several social species (Chapman and Crespi 1998; Chapman et al. 2000). In each case, a single locus proved to be suitably polymorphic for all species studied (Table 2). However, one population of *K. xiphius* for which inbreeding values were measured in two different years was polymorphic at three loci. After a bushfire, another collection from this population revealed that only one of the three loci remained polymorphic, perhaps evidence of a population bottleneck. For *K. antennatus*, we have two localities and we estimate inbreeding and present these separately (Table 2). However, the estimate used in the ancestral analysis (Figure 1) was computed with a combined data set with the two demes (localities) labeled. The inbreeding estimates for the social taxa are from published sources but are again presented at the level of locality (locality = subpopulation). Where data were collected from multiple localities (*Kladothrips hamiltoni*, *K. morrisi*, *K. intermedius*, and *Kladothrips habrus*), an average of these estimates was presented (Figure 1) and used in our ancestral calculations. For *K. xiphius*, we collected from one locality but from different years. These are presented as separate estimates (Table 2). We were disinclined to combine these data sets as an overlapping of generations, a second (beside nonrandom mating) and potentially confounding violation of the Hardy-Weinberg principle. For the ancestral analysis we use the most recent estimate for *K. xiphius* only (Figure 1), but the substitution of the earlier estimate, or an average, does not substantially alter the outcome of our ancestral estimates. We used polymerase chain reaction (PCR) to amplify the microsatellite loci; each 15- μ l PCR mixture consisted of a final concentration of 1× buffer (Roche Diagnostics, Basel, Switzerland), 2.5 mM MgCl₂; 1.0 mM deoxynucleoside triphosphates; 0.4 mM of primer pairs; 0.6 μ l of bovine serum albumin; 1.0 μ l of an unknown concentration of template DNA; and 0.5 U of AmpliTaq Gold (Roche) polymerase enzyme. All PCR was performed using an Eppendorf Gradient 96 Plate cycler. A "Touchdown" PCR protocol that reduces annealing temperature cycles between elongation stages was used. The polymerase enzyme required an initial incubation period of 2 min at 92°C. The denaturation temperature was 92°C for 30 s followed by annealing for 45 s at 68°C. The annealing temperature was reduced by 3°C for each of seven cycles, until at 47°C, with each cycle commencing with a 30-s denaturation period at 92°C and finishing with a 1-min elongation period at 72°C. The Touchdown stage was followed by 40 cycles of denaturation for 30 s at 92°C, annealing for 45 s at 47°C, and elongation for 1 min at 72°C. The protocol was completed with an 8-min elongation

Table 2. F_{is} estimates and other result details of adult female dispersers of four nonsocial gall-forming thrips

Species	Primers	F_{is} estimate (SE)	No. galls/ No. individuals	Allele lengths [frequency of the most common allele]
<i>Kladothrips rugosus</i> ^(s)	rugAAT1	0.09 (0.25)	21/21	121, 124 [0.71]
<i>Kladothrips rugosus</i> ^(r)	rugAAT1	0.59 (0.16)	27/27	134, 137 [0.63], 140, 143
<i>Kladothrips xiphius</i>	rugAAT1	0.68 (0.22)	12/12	249, 255 [0.67]
<i>Kladothrips xiphius</i> ^(C)	rugAAT1 xipAAT1 xipAAT2	0.65 (0.11) 0.65 (0.69) 0.49 (0.41)	24/24 24/24 —	230–296, 255 ^a [0.38] 140 [0.83], 143, 162 128, 134, 137, 143 [0.65]
<i>Kladothrips arotrum</i>	aroAAT1	0.54 (0.15)	15/66	148, 151 [0.41], 152, 154, 157
<i>Kladothrips antennatus</i> ⁽¹⁾	antAAT11	0.50 (0.48)	22/91	258, 276 [0.97], 294
<i>Kladothrips antennatus</i> ⁽²⁾	antAAT11	0.59 (0.27)	10/17	276, 288, 294 [0.80], 297

Primer pair sequences used to amplify microsatellite loci in nonsocial gall-forming thrips: rugAAT1, (+) 5'-ACG AGT TTT TGG GAT TGT A-3' and (-) 5'-AAC CCG CAC AGA AAA TAG ACG-3'; antAAT11, (+) 5'-GCT TCA GGT TCG CCA TTG TC-3' and (-) 5'-CCT TTT ACT TTC ATT GTT TTT-3'; aroAAT1, (+) 5'-GCG TTT CCA TTA CAG AGA CCA-3' and (-) 5'-GGC TGA AGA GGA GAA GGT GGA-3'; xipAAT1, (+) 5'-GCC CTT TCA ACT CTC GCA CAC-3' and (-) 5'-GGC TGA AGA GGA GAA GGT GGA-3'; xipAAT2, (+) 5'-AAA CTT TAC TCG GTC GGT AGC-3' and (-) 5'-GTT GTC TCC CCA CGT TGG ATC-3'. There is an additional linker sequence at the 5'-end of each positive primer—5'-TGT AAA ACG ACG GCC AGT-3'. The sample size, no. individuals, is the number of individual adult female thrips assayed. The number of alleles scored for each locus and base-pair lengths of the alleles belonging to each locus for each subpopulation are given. The population *K. xiphius*^(C) had 13 alleles ranging from 230 to 296 bp. (s), smooth gall morphology; (r), ridged gall morphology; (C), collected in 2000; (1), site 1; (2), site 2. An inbreeding estimate for *K. xiphius*^(C) over three loci is bracketed. Standard errors are indicated in parentheses and were estimated by jackknifing across groups and across loci for *K. xiphius*^(C).

^aThe 255-bp allele was the most common allele in both populations of *K. xiphius*.

period at 72°C followed by a 10-min cooling stage at 4°C. This PCR product was then used as a DNA source in a fluorescent dye (FAM, 6-carboxy-fluorescein)-labeled PCR, as described by Schuelke (2000). A short linker sequence (5'-TGT AAA ACG ACG GCC AGT-3') was added during the manufacturing of one primer in each pair (Table 1, as in caption of Figure 1) that complemented an FAM-labeled oligo. This oligo was added to the second PCR to produce a fluorescently labeled product for genotyping. Genotyping was performed using an ABI Prism 377 DNA Sequencer at the Institute of

Medical and Veterinary Science in Adelaide, South Australia. Inbreeding, F_{is} , was calculated using RELATEDNESS 4.2b (Goodnight and Queller 1994).

An overestimation of levels of homozygosity can arise from two sources of error: (1) nonamplification of an allele associated with a change to its primer site (null alleles) and (2) incorrect identification of a haploid male as a diploid female. In haplodiploid populations null alleles can be detected by the nonamplification of male haplotypes. Male offspring should show nonamplification if the mother carries a null

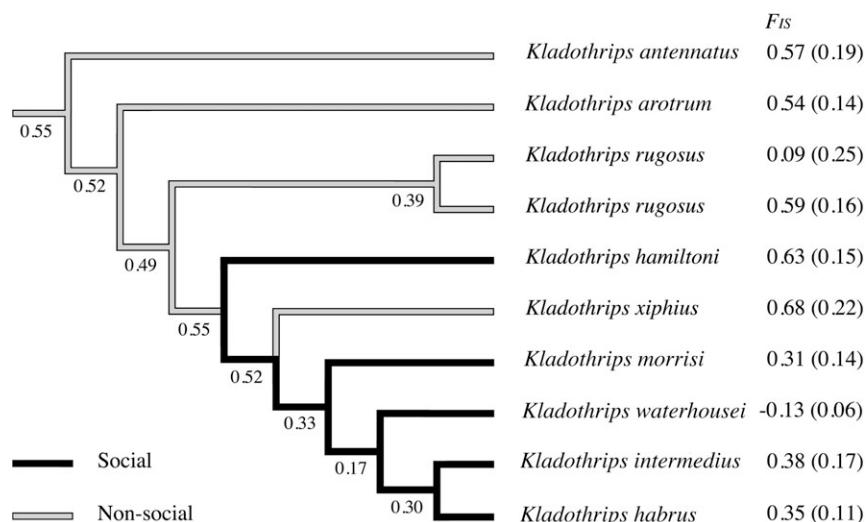


Figure 1. The evolution of foundress inbreeding levels in Australian gall thrips. Inbreeding estimates [F_{is} (SE)] for extant taxa were calculated using RELATEDNESS 4.2. Ancestral inbreeding values were inferred using COMPARE 4.6. Inbreeding levels were high at the origin of soldier castes and declined along the node leading to (*Kladothrips waterhousei* + *Kladothrips habrus* + *Kladothrips intermedius*).

marker. None of the males analyzed in this study showed evidence of such processes.

Inference of Ancestral States

The F_{is} measure was used to estimate inbreeding at the level in individuals in reference to the subpopulation (or geographic locality). To infer ancestral values of inbreeding, we used the generalized least squares statistical model in COMPARE 4.6 software (E. Martins, University of Indiana, <http://compare.bio.indiana.edu>). Phylogenetic relationships for the Australian gall-forming *Acacia* thrips have been well resolved (Crespi et al. 1998; Morris et al. 2001). Branch lengths were set to be equal, which represents a speciation model of evolutionary change (changes coincident with speciation events, not time), and as no branch length information was available for the putative *K. rugosus* species added to the previously estimated phylogeny. Analysis using branch lengths based on inferred divergence in the mitochondrial cytochrome oxidase I gene (a gradualistic model) yielded nearly identical inferences.

Results

Inbreeding (F_{is}) estimates for nonsocial thrips ranged from 0.09 to 0.68, with the lowest values in *K. rugosus* (smooth-gall form) and the highest values in *K. xiphius* (Table 2). Estimates for the *K. xiphius* collections sampled 2 years apart produced similar results of $0.63 \pm SE 0.06$ (2000) and $0.68 \pm SE 0.22$ (2002). Despite evidence of a population bottleneck, both inbreeding estimates appear to concur. The *K. antennatus* collected from two sites 93 km apart exhibited high inbreeding for both populations ($0.50 \pm SE 0.48$ and $0.59 \pm SE 0.27$). The near-equivalent values of inbreeding estimates found in these temporally separated samples of *K. xiphius* and the spatially separated samples of *K. antennatus* provide support to the hypothesis that inbreeding is a species-specific trait.

Inbreeding values for *K. rugosus* differed between the thrips that inhabited galls of the two different forms, smooth ($F_{is} 0.09 \pm SE 0.25$) and ridged ($F_{is} 0.59 \pm SE 0.16$), despite these different galls being collected from the same trees. Indeed, the populations, as defined by the morphology of the gall, do not share any alleles in common (Table 2). The populations thus appear to be reproductively isolated and we considered them as two different species. Furthermore, close inspection has revealed morphologically fixed characters that distinguish these populations (taxonomic description in preparation).

To estimate ancestral states of inbreeding, F_{is} values for social (Chapman et al. 2002) and solitary species were mapped onto a phylogeny of Australian gall-inducing thrips (Figure 1). From the resultant phylogenetic pattern, we infer that high levels of inbreeding were present prior to and during the origin of soldiers. Overall, inbreeding levels were relatively high at the more basal nodes, and they decreased to the lowest estimated inbreeding values in the clade comprising *Kladothrips waterhousei*, *K. inermedius*, and *K. habrus*. The last two species also show the most extreme reproductive division

of labor of all the eusocial gall-inducing thrips, in that soldiers produce few or no offspring (Chapman et al. 2002).

Discussion

The inbreeding estimates presented here are the first for non-social gall-inducing thrips species. Foundress inbreeding levels were high (F_{is} of 0.54 and above) with the sole exception of the smooth-gall morph form of *K. rugosus*. These inbreeding values, combined with previous estimates for social species (Chapman et al. 2000, 2002) indicate that high levels of inbreeding were present prior to the origin of soldiers. Thus, soldier behavior and morphology apparently evolved under a population-genetic structure of partially inbred foundresses, and such inbreeding is expected to have influenced the probability of altruism reaching fixation within this lineage.

High F_{is} levels of foundresses in nonsocial gall thrips are due to sibmating, presumably prior to dispersal, between winged male and female brood. This mating system is concordant with the highly female-biased brood sex ratios of species like *K. antennatus* and *K. arotrum* (Crespi 1993), which also reflect strong local mate competition. In comparison to these two species, *K. rugosus* probably exhibit higher levels of mating after dispersal because their galls are often founded by a female and a male together (Crespi 1992a).

How might inbreeding have been involved in the origin of soldiers in gall thrips? Soldiers evolved from foundress offspring that became adults within the gall, did not disperse, and began to express defensive behavior against *Koptothrips* kleptoparasites in this new selective context (Crespi and Mound 1997; Crespi et al. 1997). The evolution of this morph would have required that the benefits of staying within the natal gall, which involved avoidance of dispersal costs, some degree of direct reproduction, and enhanced gall defense, exceed the benefits of dispersal and independent reproduction.

Female protosoldiers would have only been capable of mating with brothers. In such populations, the dispersing brood would be produced by both the foundress and the protosoldiers, and the origin of soldiers would involve the insertion into the life cycle of a partial generation of sibmating (Crespi et al. 1997). Population-genetic modeling has shown that such soldier sibmating, coupled with some degree of foundress outbreeding, sets up an across-generation oscillation in inbreeding levels, as foundresses become more inbred than soldiers (Chapman 2003; Chapman et al. 2000). This oscillation can favor the evolution of natal philopatry and helping behavior by protosoldiers. Thus, if protosoldiers were to disperse (and outbreed), they would suffer lower average relatedness to their own offspring than if they remained at home (and sibmated). This inbreeding-outbreeding oscillation effect would have been augmented by the high levels of female soldier reproduction inferred for lineages near the origin of soldiers (Chapman et al. 2002; Kranz et al. 2001).

Under the scenario described earlier, partial inbreeding can influence the origin and/or form of soldiers in four ways. First, complete sibmating by protosoldiers, combined with partial sibmating by foundresses, elevates levels of within-gall

genetic relatedness. Such higher relatedness can facilitate the evolution of helping via kin selection (Chapman et al. 2000). Second, inbreeding reduces the haplodiploidy-induced relatedness asymmetries that have been hypothesized to favor female-biased helping (Chapman et al. 2000; Hamilton 1972). This effect is consistent with the presence of soldiers of both sexes in gall thrips. Third, male protosoldiers benefit from staying and helping under sibmating because they are related only to daughters ($r = .5$) if they disperse, but if they reproduce and help in their natal galls, they gain inclusive fitness via daughters ($r > .5$), sons of sisters ($r = .25$), nieces ($r > .25$), nephews ($r = .25$), brothers ($r = .5$), and sisters ($r = .25$). Finally, as noted above, natal philopatry by protosoldiers should be most strongly favored when protosoldier females that dispersed would outbreed, such that they would exhibit relatively lower average relatedness to offspring than do the protosoldiers who remain within their natal gall and sibmate. The most pronounced inbreeding-outbreeding oscillations would therefore be generated when foundresses outbreed. By our inferences, foundresses of the solitary ancestors of social species exhibited a mixed mating system of partial outbreeding (with F_{is} about 0.5), which means that inbreeding-outbreeding oscillations were present but muted at the origin of protosoldiers but still favoring their evolution to some degree. Taken together, these four considerations suggest that inbreeding was important for both the origin and the bisexual nature of helping in Australian gall thrips.

Subsequent to the origin of soldiers, levels of sibmating by foundresses tend to decline (Figure 1). Coincident with these lower levels of inbreeding is an increase in the strength of reproductive division of labor, leading to the most altruistic soldiers, with the lowest levels of soldier reproduction, in *K. intermedius* and *K. habrus* (Chapman et al. 2002). Decreased levels of sibmating by foundresses may have facilitated the evolution of more extreme reproductive division of labor because they increase the size of the oscillations in inbreeding values between the two generations. This increase would lower the threshold for defensive specialization (Chapman 2003) and thus allow the evolution of reduced soldier reproduction without the loss of soldiers. Here, increased skew is favored directly by the trade-off between defensive ability and soldier reproduction such that less soldier reproduction engenders more effective defense (Perry et al. 2003; Simpkins 2002).

In contrast to the increased specialization of soldiers in *K. intermedius* and *K. habrus*, the species *K. xiphius* has lost the soldier caste. The foundress inbreeding level in this species ($F_{is} = 0.67$) is high compared to those of related social species other than *K. hamiltoni* (Figure 1). However, inbreeding levels do not appear to have undergone substantial change along the lineage leading to this species. We postulate that ecological factors, perhaps related to the distribution of *K. xiphius* in north-central Western Australia, where no species with soldiers at all are found, are responsible for this loss of sociality.

How does the interplay between inbreeding and sociality in Australian gall thrips compare to that in other animals? Among termites, inbreeding-outbreeding cycles directly parallel to those in social gall thrips have been suggested as an important factor favoring the origin of helping (Bartz 1979;

Flesness 1978; Hamilton 1972; Tyson 1984). By this hypothesis, neotenic offspring of a founding male-female pair sibmate for one or more generations and produce inbred alate dispersers that outbreed and found new colonies; this breeding scheme can generate protoworkers that are more closely related to their sibs than to their own offspring. A notably high proportion of species in the phylogenetically "primitive" termite families Mastotermitidae and Termitidae (Eggleton 2001; Thompson et al. 2000) produce neotenes (Abe 1991; Higashi et al. 2000), and some, such as *Mastotermes*, exhibit significant inbreeding levels (Goodisman and Crozier 2002). However, the degree to which even these termites and others fit all the demographic requirements of the inbreeding-outbreeding hypothesis is still unclear (Myles 1988; Myles and Nutting 1988; Thorne 1997).

Among ants, inbreeding is most common in socially parasitic species (e.g., Bourke and Franks 1995; Buschinger 1989; Hölldobler and Wilson 1990). However, it has also been reported in species with mass nuptial flights, such as *Pogonomyrmex occidentalis* (Cole and Wiernasz 1997) and *Formica exsecta* (Sundström et al. 2003), and it can be inferred from various species with wingless, fighting males (Hölldobler and Wilson 1990, p. 185). Inbreeding does not appear to be common in morphologically primitive ant species (Hölldobler and Wilson 1990, p. 29), but a robust test for a role of inbreeding in the origin of ants will require more accurate and complete phylogenies (Baroni-Urbani et al. 1992) and more extensive population-genetic studies of putatively primitive species.

In spiders, inbreeding is tightly linked to sociality, as almost all the most cooperative species are highly inbred (Avilés 1997; Evans and Goodisman 2002; Henschel et al. 1995; Johannessen et al. 2002; Riechert and Roeloffs 1993; Smith and Hagen 1996). These "permanently social" spiders are apparently subject to strong ecological constraints on independent colony founding that have led to the suppression of dispersal and reproduction only by budding (Avilés 1997). The origins of such obligate sociality were apparently associated with transitions from outbred to inbred mating systems (Avilés 1997). We suggest that, as in thrips and termites, such transitions may have been facilitated by cycles of inbreeding punctuated by occasional outbreeding, which could have generated relatedness asymmetries enhancing philopatry and favoring increased levels of cooperation (see also Avilés and Gelsev 1998).

Among vertebrates, the main effect of inbreeding on the evolution of sociality appears to be a role for incest avoidance in disfavoring offspring reproduction (Bennett et al. 1999; Cockburn 1998; Cooney and Bennett 2000; French 1997; Getz and Carter 1998; Greef and Bennett 2000; Solomon and Getz 1997). Adult offspring who remain on the natal territory with their mother or parents may thus forgo breeding in part because only close relatives are available as mates, and inbreeding depression would greatly reduce the benefits of personal reproduction. The costs of inbreeding depression in social vertebrates can apparently only be overcome via severe ecological constraints on dispersal and independent reproduction, coupled with strong benefits from group living and cooperation. This combination of costs and benefits appears to have

evolved only in naked mole rats, which exhibit some inbreeding, within-group relatedness on the order of 0.80, and extremely high constraints on successful dispersal (Bennet and Faulkes 2000; Burland et al. 2002).

Taken together, these examples of inbreeding in diverse social animals suggest a synergism between ecological constraints and mating systems. Thus, at least in thrips, termites, spiders, and naked mole rats, especially strong constraints on independent reproduction apparently led to inbreeding, which elevated within-group relatedness, generated relatedness asymmetries, or enhanced inequalities in reproductive capacity (Brown and Pimm 1985). In turn, these relatedness and reproductivity effects would have increased the inclusive fitness benefits accrued from helping. Unraveling the effects and causes of inbreeding in social evolution will require comparisons between populations or closely related species or further phylogenetic studies that link inbreeding levels to variation in social behavior and social systems.

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