The Evolutionary Ecology of Eusociality in Australian Gall Thrips:

a 'Model Clades' Approach

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Summary

We integrate phylogenetic information with data on genetic relatedness, inbreeding, sex ratios, reproductive skew, host-plant use, gall morphology, soldier defensive behaviour, kleptoparasite pressure, and demography to evaluate hypotheses for the origin and evolution of soldier castes in Australian gall thrips. Necessary and sufficient conditions for the single origin of thrips soldiers appear to include high relatedness and inbreeding, strong kleptoparasite pressure, small brood size, and long duration of the gall. However, only brood size and gall duration apparently changed (becoming smaller) concomitant to the origin of soldiers. Reproductive skew between the foundress and soldiers was relatively low at the origin of soldiers, but increased substantially along the lineage leading to two species, *K. habrus* and *K. intermedius*. These two species also exhibit a relatively high propensity for defense by soldiers. Analysis of the associations between genetic and ecological traits that resulted from the social-adaptive radiation of gall thrips with soldiers provides two main insights. First, fewer matings by foundresses, and less mating after dispersal, result in stronger local mate competition, higher relatedness (and a higher inbreeding coefficient) among soldier females, and a stronger female bias in dispersers. Second, gall size apparently constrains the reproduction of soldiers, with less soldier reproduction favoring the evolution of more-effective, more-altruistic soldiers. Moreover,

when soldiers are more effective, fewer of them can be produced, leading to higher production of dispersers. Soldiers were apparently lost in two lineages, in both cases in conjunction with a shift to a phylogenetically-divergent species of *Acacia* host plant. Our analyses demonstrate that the evolution of soldiers in thrips is driven by a combination of selective pressures at three levels, from host-plant, to conspecific interactions, to kleptoparasites.

Introduction

The evolution of eusociality is one of the classic problems in evolutionary biology, yet it has seldom been addressed in an explicitly phylogenetic context. To analyze the evolution *per se* of social behaviour, data on the diverse genetic, phenotypic and ecological causes of social system variation should be analyzed via inferences on a species-level phylogenetic tree. For most social insects, the origins of eusociality are so deep in antiquity that such studies are difficult or impossible (Holldobler and Wilson 1990; Thorne 1997; Danforth 2002). However, some groups, such as the gall-inducing *Acacia* thrips, appear sufficiently young that robust inferences can be made regarding how their social systems have been assembled by selection (Crespi et al. 1998, Morris et al. 2001; Crespi et al. 2004).

Australian gall-inducing thrips are small insects that produce a domicile in developing phyllodes (petioles modified to serve as both stem and leaf) on *Acacia* trees. Gall induction is linked to the availability of new phyllode growth stimulated by episodic rainfall in arid regions, and galls are only initiated on very young phyllode tissue (Mound, 1971, 1994; Mound *et al.*, 1996). A single, foundress female, in some species accompanied by a male, initiates the gall, and once completely interred she begins laying eggs (Crespi & Mound, 1997). For 7 of the 23 described species of Australian gall-inducing thrips, the first individuals of the foundress' brood to eclose are gall-bound soldiers, which are morphologically and behaviourally specialized for defending the fully-winged dispersing brood (Crespi, 1992*a,b*; Perry et al. 2004) The galls formed by social thrips have been described as a 'factory fortress' since they provide both food and shelter for all occupants in a harsh, xeric environment (Crespi, 1994; Queller & Strassmann, 1998; Chapman *et al.*, 2002). The concentration of these critical resources in a well-defined physical structure, and the limitation of gall induction to a short temporal window, means that there is extremely strong selection to defend or hide this resource against usurpers. Thrips species in the genus *Koptothrips* are specialist invaders of these galls, which kill the occupants and then breed within the gall. *Koptothrips* are thought to be a major selective force underlying the evolution of soldier morphology and behaviour (Crespi, 1996; Crespi & Mound, 1997; Crespi & Abbot 1999).

Here, we review the evolutionary ecology of soldiers in thrips, with a focus on the integration of phylogenetic information with data on genetics, phenotype, ecology, and demography. We describe a useful approach to such integration, based on the concept of 'model clades': monophyletic groups for which sufficient multidisciplinary, integrative data are available to infer and evaluate scenarios for the joint roles of intrinsic and extrinsic factors in social evolution. First, we explain this approach, and how it extends previous approaches for comparative analysis. Second, we present our current hypothesis of phylogenetic relationships among gall thrips species with and without soldiers. Third, we describe how patterns of interspecific variation in genetic relatedness, inbreeding, sex ratios, reproductive skew, host-plant use, gall size and shape, kleptoparasite pressure, and soldier behaviour have evolved, in our phylogenetic context. Fourth, we integrate these patterns and propose a scenario for the interplay of intrinsic and extrinsic forces in the origin and diversification of soldiers in Australian gall thrips. Finally, we describe salient convergences and divergences between these thrips and other social animals, to further the development of general theory for how and why social behavior evolves.

(1) A 'MODEL CLADES' APPROACH TO ANALYZING SOCIAL EVOLUTION

Most analyses of the evolution of sociality have focussed on inferring the adaptive significance of social behaviour and social systems from functional-design studies on extant species. This approach has been especially fruitful using 'model systems' (Dugatkin 2001) such as the Hymenoptera *Polistes fuscatus, Apis mellifera, Lasioglossum zephyrum*,

and the aphid *Pemphigus spyrothecae,* for which extensive studies have been performed on aspects of behavior, ecology, and molecular genetics.

Packer (1991) pioneered the use of phylogenies for the study of social evolution, whereby the numbers and patterns of origins and losses of eusociality are reconstructed for an entire clade. This approach has since been applied to diverse social taxa (Stern & Foster 1996, 1997, Danforth 2002, Crespi et al. 1997; Choe & Crespi 1997), and extended by mapping data on one or few phenotypic traits relevant to social evolution onto the phylogeny, and linking inferred social-evolutionary trajectories to their putative causes (e. g., Ross and Carpenter 1991; Faulkes et al. 1997; Hunt 1999; Chapman et al. 2002). These analyses have been extremely useful in providing evolutionary insights, but the approach is limited by a tradeoff between the number of taxa in any analyses and amount of data available (i. e., the number of variables quantified) for the full taxon complement.

In this review, we integrate the phylogenetic approach to social-behaviour studies with the concept of 'model systems', by jointly analyzing diverse sources of datain the context of a species-level phylogeny for Australian gall thrips on *Acacia*. The rationale for this methodology is that origins, losses, and other transformations in social systems take place over evolutionary times scales, and are driven by changes in myriad ecological, demographic, morphological, and population-genetic traits. Thus, robust inference of the causes of social evolution over long-term time scales requires that diverse, multidisciplinarydata be collected for an entire clade of social species and non-social relatives. Application of this 'model clades' approach is difficult because it requires a robust, more-or-less complete species-level phylogeny, combined with salient data on enough of the species for reasonable inferential and statistical power. However, such data, analyzed using ancestor-reconstruction and correlated-evolution methods (Felsenstein 1985; Harvey and Pagel 1991; Maddison & Maddison 1992; Pagel 1994; Doughty 1996), should yield a more comprehensive picture of the causes and patterns of evolutionary change in social traits than single-species studies or comparative analyses using small sets of paired variables. Here, we apply this model clades approach to deciphering the causes of the origin and diversification of sociality in Australian gall thrips by presenting the phylogenetic hypothesis for this group, inferring the ancestral traits of Australian gall thrips that are presumed to affect social evolution, and comparing across and within lineages without and with soldiers for socially-relevant traits.

(2) PHYLOGENY

A well-resolved and well-supported phylogeny for Australian gall thrips on *Acacia* has been inferred using DNA sequence from the genes elongation factor 1a, wingless, cytochrome oxidase I, and 16S ribosomal DNA (Morris et al. 2001; Crespi et al. 2004). This phylogeny includes all of the described species except one (*K. carneorum*), and it exhibits strong bootstrap support for the monophyly of the group as a whole, and for most of the nodes. The sister-group to these gall-inducers (*Dactylothrips* and *Rhopalothripoides*) also live on *Acacia*, but they are not gall-inducing, instead living in various enclosed spaces, such as old galls of other insects, or cracks in bark or stems. Some of these 'opportunistic' species exhibit wing polymorphism, and some *Dactylothrips* show forms of parental care involving defense of their domicile using tergites modified into pincers (Crespi and Mound 1997; Crespi et al. 2004). Gall-induction apparently evolved from deformation of plant material (here, phyllodes) during feeding, as in other insect groups (Shorthouse & Rohfritsch 1992; Williams 1994; Crespi et al. 1997).

The basal lineages of gall thrips, comprising *K. pilbara, K. schwarzi, K. antennatus,* and *K. zygus*, all inhabit Acacia in the highly arid zone. They induce simple tubular galls resembling tightly rolled phyllodes on Acacia species in the taxonomic sections Juliflorae and Phyllodines, and adult offspring of the foundress eclose within the galls. The three, more-derived lineages can usefully be divided into three groups based on their host-plant

relationships and life histories (Figure 1). Lineage A is found on Acacia in the taxonomic section Juliflorae, also in the highly arid zone, where they induce more or less spherical, tightly closed galls that appear nearly impervious to invasion by natural enemies such as kleptoparasites; we this refer to these species are 'hiders'. All but one species in lineages B and C inhabit *Acacia* in the section Plurinerves, mostly in semi-arid regions. The species in lineage B induce relatively large galls of diverse forms that persist for relatively short lengths of time on the host plant, and they exhibit high fecundity relative to most of the species in lineage C (Table 1). With the possible exception of *K. maslini*, these species are heavily beset by kleptoparasites in the genus *Koptothrips*, as well as other natural enemies (Crespi & Abbot 1999); we refer to them as 'fliers', since their brief within-gall phase of the life cycle appears to be an adaptation to reduce vulnerability to these enemies.

Lineage C, the 'fighters', include all of the species with soldiers, plus two species without soldiers, *K. xiphius* and *K. rodwayi*. These species tend to exhibit relatively small galls, which is associated with low fecundity, and the galls persist for a long time on the host plant (up to one year). There are two equally-parsimonious inferences for the pattern and number of origins and losses of soldiers: (1) one origin at the base of the lineage, and two losses, one each in *K. xiphius* and *K. rodwayi*, vs. (2) two origins (one in *K. hamiltoni* + *K. harpophyllae* and one in *K. morrisi* + *K. waterhousei* + *K. habrus* + *K. intermedius* + *K. rodwayi*), followed by one loss (in *K. rodwayi*) (Morris et al. 2001). Two lines of evidence support the former scenario of one origin. First, the hypothesis that the lineages leading to both *K. xiphius* and *K. rodwayi* evolved via loss of soldiers is consistent with their habitation of *Acacia* host plants that are phylogenetically highly-divergent from the *Acacia* inhabited by the other thrips species in this clade (Crespi et al. 2004). Indeed, such large-scale host plant shifts are apparently always associated with substantial phenotypic and life-historical changes in Australian gall-inducing and related thrips on *Acacia* (Crespi et al. 2004). Second, Wcislo and Danforth (1997) present evidence that ancient origins, punctuated by scattered losses, characterize the evolution of eusociality in other insect groups; this pattern may be related to the complex nature of the trait 'eusociality', such that it is evolutionarily difficult to gain (via multiple sequential steps) but easy to lose (via a change in any of a large suite of traits, at least for so-called primitively-eusocial forms) (see also Danforth 2002). The hypotheses of one vs two origins of soldiers can best be tested further via testing for homology, parallelism or convergence in the genetic or physiological underpinnings of soldier morphs. At present, we provisionally accept the single-origins hypothesis, and note that our inferences about character change are not qualitatively altered if the hypothesis is later rejected.

(3) VARIATION IN TRAITS RELATED TO SOCIALITY

(a) The Groundplan of Australian Gall-inducing *Acacia* Thrips

Three traits of the insect order Thysanoptera are especially relevant to the origin and evolution of soldiers. First, all thrips are haplodiploid (Stannard 1968; Crespi 1991, 1993; Mound and Heming 1991). As in Hymenoptera, this genetic system engenders sex ratio manipulation by mothers, reproduction by virgins, the potential for relatedness above 0.5 and relatedness asymmetries between the sexes, and a genetic tolerance to inbreeding (Chapman 2003). Second, as in aphids, the forelegs of thrips can be readily modified to serve as weaponry, as evidenced by the high frequency of male foreleg allometry and male fighting within this order (Crespi 1986, 1988, 1993). Moreover, intraspecific fighting is common in thrips (as in some aphids), and it provides the raw material for selection on interspecific defense against natural enemies (Whitham 1979; Aoki and Makino 1982; Crespi et al. 1997; Crespi & Mound 1997; Inbar 1998). Third, as in aphids, ants, and termites, wing polymorphism is common in Thysanoptera (and in *Acacia* thrips related to those that induce galls on this plant genus). Wing polymorphism can serve as a template for the evolution of soldiers, as the evolutionarily-facile reduction or loss of wings can free

up physiological resources (Roff 1986; Roff & Fairbairn 1991; Roff & Bradford 1996) for the development of weaponry such as enlarged forelegs, as well as for reproduction. Indeed, in *K. intermedius* (formerly called *Oncothrips tepperi*), soldiers show a strong negative correlation between wing length and foreleg size (Crespi 1992b).

The evolution of soldiers only in the thrips that induce galls on Australian *Acacia* can, we believe, be traced to the unique nature of the gall as a resource, and the harsh environment of the Australian interior. Thus, *Acacia* galls can only be induced during the very short temporal window when new phyllodes are being produced by the host plant, annually in many species and less frequently in areas of more-unpredictable rainfall. A gall represents food, shelter and reproduction for a lifetime, it cannot be replaced if lost, and the environment outside of the gall is among the most harsh and arid on earth. The high value of galls, and the ecological constraints associated with their induction, makes them a coveted resource for natural enemies, and soldier morphs represent one adaptive life-history strategy (in addition to a short life cycle, or a tightly-enclosed gall) for maximizing inclusive fitness in the face of such strong selection.

(b) The Origin of Soldiers

Given that Thysanoptera in general, and Australian gall thrips in particular, exhibit several crucial preadaptations to the evolution of soldiers, we are next faced with the question of why soldiers originated in one particular lineage and why they were lost in others. Addressing these questions requires inferring the states of diverse ecological, morphological, demographic, genetic and other traits at the branch subtending the origin of eusociality, as well as inference of which of these traits changed along this branch, and how. Figure 2 shows that the lineage leading to the origin of soldiers apparently exhibited a suite of traits that remained more or less constant during the transition, but apparently formed important parts of the selective context whereby soldiers evolved. These traits include: (1) fighting in foundresses, (2) a male founder being present in a substantial proportion of galls, (3) high relatedness along gallmates, and substantial inbreeding in the foundress, and (4) attack by *Koptothrips* kleptoparasites, and (5) pupation of dispersers in the soil. Thus, the context for the origin of soldiers involved the morphology and behavior of foundresses in defending against *Koptothrips* serving as an apparent preadaptation to within-gall defense by offspring. Moreover, inclusive fitness effects can be inferred to have been strong, as soldiers of both sexes were closely-related to siblings and their mother, due to inbreeding, an apparent lack of intergall mixing at any stage (from genetic and observational evidence; Crespi et al. 2004), and the presence of a male founder leading to broods being comprised of full siblings. Finally, the inferred presence of attack by *Koptothrips* during the transition to soldiers is consistent with the hypothesis that they represent one of the main selective forces for soldier evolution (in conjunction with intragall reproduction by offspring), but also that *Koptothrips* attack did not coincide with and precipitate the origin of soldiers.

Three species in the sister-group to the lineage with soldiers, *K. rugosus*, *K. ellobus*, and *K. acaciae*, and the social species *K. harpophyllae*, exhibit a substantial incidence (on the order of 50% of galls) of single male founders being present at gall initiation, a pattern evidently driven by male fighting and mate-guarding (Crespi 1992a). This mating system might also lead to split sex ratios, if some foundresses inbreed before dispersal while others mate after dispersal with a non-relative. Galls with male founders should thus exhibit single-mating by foundresses with an unrelated male, and high relatedness of brood within galls. Similarly, *K. rugosus*, a species in the sister-clade to the clade bearing soldiers, exhibits 'split' sex ratios due to a notable incidence of foundress virginity (Kranz et al. 2000). Both of these split sex ratio effects could have disproportionately favored the origin of soldiers in some subset of galls, where Hamilton's rule was satisfied (Grafen 1986; Godfray and Grafen 1988); this hypothesis can be tested further via studies of *K. harpophyllae*, the only species with soldiers and a high incidence of male founders.

The lineage giving rise to soldiers apparently exhibited large galls with many dispersers and soldiers relative to more-derived lineages, relatively high reproduction by soldiers (Chapman et al. 2002), relatively high Fis values for foundresses, among-soldier relatedness that was high but not notably so, and a relative male bias to the sex ratio of soldiers; the sex ratio of dispersers is difficult to infer with any confidence, as it differs so strikingly between *K. morrisi* (41%) and *K. hamiltoni* (6%). These inferences can be interpreted in the context of the inclusive fitness model of Chapman (2003), which elucidates the expected role of sex ratios, inbreeding and soldier reproduction in the origin of helping by male and female protosoldiers. This model predicts two main patterns that accord with the inferences from phylogenetics and character mapping. First, based on considerations of relatedness and inbreeding, the model predicts a lower soldiering threshold in males than females, which fits with the relatively high proportion of male soldiers in *K. hamiltoni* (52%) and *K. morrisi* (25%) compared to the other species, assuming that there are factors other than relatedness and inbreeding (such as sex differences in body size) that affect this trait. Second, the model demonstrates that reproduction by male and female protosoldiers favors the origin of helping, by raising the Fis of dispersers and leading to alternating Fis between generations, which can make soldiers more highly related to natal colony-mates than they would be to their own offspring. This effect represents an interesting combination of Trivers and Hare's (1976) hypothesis that female protohelper reproduction should have favored the origin of hymenopteran eusociality, and Bartz's (1979) ideas on the potential role of inbreeding/outbreeding cycles.

In general, the presence of bisexual helping on social thrips is likely to be associated with the high similarity in morphology of female and male *Acacia* gall thrips (in contrast to many other social insect taxa), with both sexes similarly-armed though females tend to be larger in overall size, and the tendency of strong inbreeding to reduce the relatedness asymmetries of haplodiploidy (Chapman and Crespi 1998). Thus, there has presumably been selection for both sexes to fight and help, and they have evolved similar armature. However, the presence of soldiers of both sexes may belie important sex differences in willingness to defend (i. e., who takes on the risky task of attacking a *Koptothrips* first)(Crespi and Mound 1997).

Coinciding with the inferred origin of soldiers were changes in four traits: (1) galls became smaller and evolved from being relatively round to flat or elongate, (2) foundresses became non-physogastric, (3) broods evolved to be smaller, and (4) galls persisted for considerably longer on the host plants (time for soldiers to eclose) (Crespi et al. 1997; Crespi and Worobey 1998; Kranz et al. 2001,a,b; Crespi et al. 2004). These four traits are closely-related and presumably evolved in concert.

Small galls are strongly associated with small broods because gall volume imposes constraints on brood size, and small broods are associated with a lack of physogastry. Given that most of the species with soldiers utilize host plants that are also occupied by congeneric gall thrips with much larger, rounded galls and without soldiers (Crespi et al. 1997, 2004), gall size cannot be intrinsically constrained by phyllode size. Instead, we propose that large-gall and small-gall species have evolved to utilize the gall resource either quickly, leading to a short within-gall life cycle (as in Kladothrips rugosus, for example), or slowly, leading to the more prolonged within-gall life cycle of species with soldiers (Crespi et al. 2004). The physiological basis for this difference is unclear, as galls grow to full size within two weeks or less and last for up to a year, but it may be related to the cumulative amount of resource that the thrips within a gall can extract from a single phyllode, and the rate at which they imbibe plant cell contents.

The pattern of longer-lived galls in species with soldiers suggest that these species have much lower within-gall population growth rates, which theoretical models predict should

favor the evolution of soldier defense in the ecologically-similar gall aphids (Akimoto 1996; Stern and Foster 1996). High colony longevity, which is also found in aphid species with soldiers (Moran 1993; Foster and Northcott 1994; Rhoden and Foster 2002), may have favored the origin of soldiers because galls are thereby vulnerable to natural enemies for longer periods. Longevity might also be a consequence of soldiers, whose presence could allow galls to persist longer in relative safety; however, species with soldiers do not exhibit lower rates of successful *Koptothrips* parasitism, apparently because *Koptothrips* often invade before soldiers eclose, and defense by soldiers is only partially effective (Crespi and Abbot 1999). A pattern of high parasite pressure favoring the origin and maintenance of sociality has also been proposed for some Hymenoptera (Lin 1964; Lin and Michener 1972; Kukuk et al. 1989), and other social taxa (Crespi and Choe 1997).

Species with soldiers also tend to induce relatively flat or elongate galls, which, coupled with their small size, generates a relatively high inner surface area to volume ratio. Such gall morphologies are presumably related to the space available for feeding inside, relative to the number of inhabitants. High inner gall surface area to volume ratio is also found in the galls of two gall thrips that are unrelated to the species with soldiers and their sister-group: *Kladothrips sterni* on *Acacia aneura*, and *Iotatubothrips crozieri* on *Casuarina*, both of which undergoes multiple generations in the gall. A clear relationship between multiple generations and high inner gall surface area has thus evolved convergently at least three times (Crespi and Worobey 1998), which suggests that space for feeding is related in some way to gall lifespan. Indeed, these patterns suggest strong causal connections between thrips demographics, life history, gall morphology and physiology, and soldiering. Stone and Schönrogge (2003) describes analogous adaptations of gall morphology to demography and defense, in diverse species of insects.

Understanding the connections between gall morphology, demography, ecology and behavior requires further study, especially as it appears to involve low fecundity and enhanced adaption for defense from natural enemies. As an apparent exception that supports the rule, *K. morrisi*, one of the few gall thrips with soldiers that is the only gall-inducer on its host plant, exhibits a suite of life history traits that combines elements of the soldiering species (i. e., soldiers and an elongate gall) and their significantly more fecund, solitary sister-lineage (i .e, large galls and large brood) (Kranz et al. 2001a).

(c) Diversification among Species with Soldiers

How and why, in the lineages with soldiers, has diversity in socially-salient traits such as relatedness, inbreeding, life cycle, sex ratios, reproductive skew, and soldier behavior evolved?

(i)   Inbreeding and Relatedness

Population-genetic (microsatellite) data have shown that inbreeding is common in the gall-inducing thrips on *Acacia* both with and without soldiers, however, its strength diverges considerably among these species (Chapman and Crespi 1997, Chapman et al. 2000, McLeish et al. in review, Table 1). Elevated levels of relatedness have also been detected in many social species (Chapman and Crespi 1998, Chapman et al. 2000). These estimates of inbreeding and relatedness, 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, and that high levels of inbreeding emerged well before the evolution of a sub-fertile caste (Figure 2). Character optimization indicates that the ancestral population that gave rise to soldiers may have had an average relatedness among soldiers, males and females taken together, as high as 0.78 and an inbreeding

coefficient of 0.58 (single origin of soldiers hypothesis, Chapman *et al.* 2000), and subsequently, these values evolved to be lower in more-derived social lineages. An ancestral lineage with such high levels of inbreeding would have had greatly reduced relatedness asymmetries, which.. casts considerable doubt on the importance of haplodiploidy-induced relatedness asymmetries in thrips social evolution. However, the gall-forming thrips do provide a concrete example of an inbreeding population giving rise to social lineages, and, as discussed below, inbreeding has apparently impacted the origin and forms of social behaviour.

(ii) Soldier Reproduction and Gall Morphology

Crespi (1992b) reported the first evidence that the wing-reduced individuals in thrips induced *Acacia* galls were defensive specialists. Crespi (1992b) also determined through dissections that these defensive specialists were sexually mature and perhaps capable of reproducing within the gall, but he surmised that their reproductive output was likely to be limited by available space within the gall. Chapman et al. (2002) largely confirmed this assertion by estimating the degree of reproductive differentiation between foundresses and soldiers in multiple populations of five species of gall-inducing thrips using microsatellite data and ovarian dissections.

Microsatellite-based species estimates of average per capita reproductive output of soldiers relative to the foundresses ranged from 0.005 to 0.64. Estimates were based on one or two microsatellite loci only. The paucity of polymorphic loci, likely due to population bottlenecks from frequent bush-fires and droughts in the Australian outback, and the large amount of inbreeding present in many of these species meant that conventional maternal analysis using codominant markers was not possible. Instead, estimates were obtained by assessing the difference in inbreeding coefficients as measured in soldiers and in dispersers. A mathematical relationship was developed that allowed the inference of soldier reproduction from the expected increase in the inbreeding coefficient, as measured in the dispersers, that was likely due to soldier production of female dispersing offspring. Males are not useful for estimating observed homozygosity levels and therefore were not taken into account in these estimates. Therefore, an underestimation of soldier reproductive output is expected if soldiers produce a greater proportion of males compared to that of the foundress. Soldier breeding has been shown theoretically to alter the probability of genes in colony founders being transmitted to the dispersing generation through male and female soldiers (the 'reproductive value' of males and females) from that expected for a haplo-diploid colony with no reproduction by helpers (Chapman 2003). This proposed change in reproductive value of male and female offspring leads to the expectation that female soldiers would produce a less female biased sex ratio compared to their foundress mothers. The second method utilized here, ovarian dissections, is not impacted by the sex ratio produced but simply indicates the potential for egg laying between soldiers and her foundress mother. These estimates based on ovarian dissections ranged from 0.17 to 1.1. Given that these potential eggs may not all translate into actual offspring produced, these estimates may represent overestimations of soldier reproduction. However, both approaches reveal similar variation in reproduction by the soldier group across species, and there is a strong, significant correlation between the microsatellite and ovarian-dissection estimates (Chapman et al. 2002).

The mapping of the microsatellite-based and ovarian-dissection-based estimates of relative soldier reproduction showed the same phylogenetic pattern, that levels of soldier reproduction were apparently higher in three more-basal lineages than in the two most derived lineages (Chapman et al. 2002). The inference is that thrips soldier morphology and behaviour likely evolved in the presence of considerable levels of

soldier reproduction. This scenario is consistent with philopatric offspring developing traits such as enlarged forelimbs and evolving into defensive specialists that only later evolved lowered fecundity. Correlated with this lowered reproductive output by soldiers was a shrinking of the average volume of a gall (r^2=0.89, p<0.05, using independent contrasts, Wills et al. 2004). These observations are consistent with gall volume constraining the reproductive output of soldiers, as Crespi (1992b) anticipated.

The progenitor of the modern thrips soldier appears likely to have been a non-dispersing morph that reproduced via sib-mating within its natal gall. With the subsequent loss of some direct reproductive output, the evolutionary maintenance of the developing soldier caste provides somewhat of a conundrum. The impact of the reproductive output of a proto-soldier on soldier evolution was investigated using an inclusive fitness model (Chapman 2003). Parameters in the model included mixed mating, a female biased sex ratio, and proto-soldier reproduction and the impact of these parameters on the relatedness between the potential soldier and its gall mates. In general, the results of the model indicated that allowing for some proto-soldier reproduction increased the likelihood of the evolution of a soldier caste for both female and male proto-soldiers. The main impact was that proto-soldier reproduction increased the inbreeding coefficient of the disperser generation over that of the proto-soldiers, resulting in alternating levels of inbreeding between generations. This oscillation in inbreeding coefficients favors soldiers by increasing the relatedness of a proto-soldier to natal colony-mates, relative to the relatedness it would have to its own offspring. However, the amplitude and the predicted impact of these oscillations on the evolution of soldiering are reduced as sib-mating increases within the dispersing generation. Thus, a high level of inbreeding by the foundress reduces the difference in the inbreeding coefficients between the dispersers and proto-soldiers and reduces relatedness differences for helping vs reproducing. High inbreeding and high levels of female soldier reproduction at the origin of soldiers, and the subsequent reduction of both traits in the two most derived species of gall-inducers, are consistent with the model in that inbreeding by foundresses may retard the evolution of extreme levels of altruism.

For male proto-soldiers, inbreeding by the foundress and proto-soldier reproduction both act to decrease the helping threshold. That is, when inbreeding by dispersers is high and proto-soldiers produce some of the dispersing generation, male proto-soldiers are more likely to find themselves in a position where an altruistic act within there natal nest may have a greater inclusive fitness payoff than the pursuit of direct fitness alone. As discussed below, the effects of inbreeding on male and female helping thresholds have important implications for the evolution of soldier sex ratios.

(iii) Soldier Defensive Ability

In *Acacia* gall thrips, the transition from a solitary to social life history also included a reduction in gall volume and a consequent reduction in disperser numbers, as well as an elongated period for the development of dispersing brood (Wills *et al*. 2001, 2004; Crespi et al. 2004). Thus, gall-inducing thrips lineages have evolved to adopt either of two strategies under selective pressure from *Koptothrips* invasions: (1) produce large galls and fast developing brood in order to ameliorate losses due to *Koptothrips* invaders, or (2) produce small galls, and therefore smaller dispersing brood numbers, but increase the survival of these galls by producing a specialised defending caste (the soldiers) (Crespi et al. 2004). The second strategy, defence of galls by soldiers, involves a trade-off between the number of dispersers produced and survival of the gall until dispersal. The soldiers themselves appear to make an additional trade-off. Thus, the reproductive output of thrips soldiers ranges widely (Chapman et al. 2002), and soldier defensive efficacy appears to be negatively correlated with the degree to which soldiers reproduce (r = -0.88, P = 0.0489, N = 5 by simple correlation; r = -0.84, P = 0.076 using independent contrasts Perry *et al*.

2002, Perry *et al*. 2004). Given evidence for a relationship between soldier reproduction and defensive ability, the mechanism and source of the trade-off remain unclear. Crespi (1992b) showed that in one species, *Oncothrips tepperi* (now *K. intermedius*), soldiers exhibit an inverse relationship between wing length and foreleg (weaponry) size, which is consistent with a developmental trade-off between defence and dispersal. Such a tradeoff could have helped to favor the origin of thrips soldiers by making within-gall philopatry more beneficial energetically; in other insects, reduced wings tend to be associated with increased fecundity (Roff 1986). However, the relationship of wing length and foreleg size with reproductive output in thrips is not yet known.

Tradeoffs between direct reproduction and benefits to kin via defense, such as the ones discussed here in *Acacia* gall thrips, have also been fundamental to the evolution of 'advanced' eusociality in Hymenoptera and Isoptera (Bourke and Franks 1995; Thorne 1997; Chapman et al. 2002). In thrips, further tests of the relationships between reproductive skew, soldier behavior, and other variables require the addition of data from more species to alleviate the low statistical power currently available (e. g., there are over 12 apparent sibling species in the *K. waterhousei* complex; Crespi et al 2004), as well as comparative analyses that include additional variables, such as weapon morphology and degree of gonadal development

(iv) Evolution of Sex Allocation, Ecology, Genetic Structure and Sociality

Theories linking sex allocation with the origins and forms of eusociality have long held a central and controversial role in the debate over the causes of the evolution of social behavior (Hamilton 1972, Trivers and Hare 1976, Charnov 1978, Seger 1983, Grafen 1986, Godfray and Grafen 1988; Crozier & Pamilo 1996; Hardy 2002). Australian *Acacia* thrips are especially useful for addressing these links because thrips are the only taxon other than Hymenoptera exhibiting both eusociality and facultative sex ratio manipulation via haplodiploidy. The main factors predicted to affect the evolution of *Acacia* gall thrips sex allocation include:

(1) local competition between relatives with the gall, especially competition between males for mating with sisters leading to female-biased sex allocation;

(2) levels of relatedness and inbreeding, which determine the ratio of male to female offspring that maximizes inclusive fitness of the female controlling allocation; these values are set by numbers of matings by females, the extent to which these matings take place before and after dispersal from the gall, and the extent to which soldiers reproduce; and

(3) any conflict between foundresses and female soldiers over sex allocation ratios, which is analogous to queen-worker conflict over sex allocation in Hymenoptera.

We can analyze these selective pressures using comparative data from five of the seven described species with soldiers on gall sizes, numbers of soldiers and disperers, disperser sex ratios, soldier sex ratios, relatedness, inbreeding coefficients of foundresses and soldiers, levels of reproductive skew between foundresses and soldiers, soldier propensity to attack *Koptothrips*, and soldier efficacy in killing these enemies (Table 1). The variables show marked variation among these five species. The patterns of correlation between traits demonstrate that their causal connections fall into two main clusters: (a) effects of mating system and relatedness on sex allocation (Figure 3), and (b) effects of ecology on sociality, which appear to be more or less independent of sex allocation (Figure 4).

Mating system effects on sex allocation can be inferred from the associations between disperser and soldier sex ratios, disperser and soldier inbreeding coefficients (Fis), and relatedness between female soldiers (Figure 3). Three main patterns are evident: (1) higher relatedness between soldiers is strongly associated with more female-biased

disperser sex ratios, (2) disperser and soldier Fis values are strongly positively correlated, and (3) foundress and soldier Fis values are positively correlated with soldier sex ratio. A strong though non-significant positive correlation is also apparent between female soldier relatedness and soldier sex ratio, and soldier and disperser sex ratio appear to be negatively correlated across species. Taken together, these results suggest that fewer matings by foundresses, and less mating after dispersal, result in stronger local mate competition, higher relatedness (and a higher inbreeding coefficient) among soldier females, and a stronger female bias in dispersers. Indeed, in the two cases where there is an unbiased disperser sex ratio, inbreeding among dispersers has not been detected (*O. waterhouesi,* Chapman et al 2000) or multiple mating has been implicated in lowered relatedness (*O. habrus,* Chapman & Crespi 1998).

Reproduction by soldiers varies considerably among species, and it complicates the expected relationships between inbreeding, relatedess and sex allocation. If female soldiers reproduce relatively little (as in *K. habrus* and *K. intermedius*), then soldiers and dispersers are genetically more or less equivalent, with an Fis determined by foundress mating patterns. By contrast, substantial reproduction by soldier females, which necessarily involves sibmating, raises the Fis of dispersers and creates inbreeding/outbreeding oscillations that may have favored the origin and evolution of helping (Chapman 2003); indeed, such oscillations appear to have been retained in *K. hamiltoni* and *K. morrisi*, the two most-basal species with soldiers (Table 1). In addition, the relative mating success of soldier and disperser males may differ; given partial bivoltinism (Seger 1983) soldier males may mate with both soldier females and disperser females, whereas disperser males eclose so late that they could only produce offspring via mating with disperser females.

Sex ratios vary among species, and they also exhibit a temporal variation, as one sex can be produced before the other. Among the social species studied in detail thus far, there are notable associations between the order of eclosion by sex in soldiers and three variables: soldier sex ratios, offspring sex ratios, and the extent of reproductive skew between female soldiers and foundresses (Kranz et al., 1999, 2000, 2001a,b; Chapman et al. 2002). Thus, *K. habrus* and *K. waterhousei* exhibit protogynous (females first) soldier production, strongly female-biased soldier sex ratios, an unbiased sex ratio of reproductive dispersers, and relatively high skew. By contrast, *K. hamiltoni* exhibits the opposite pattern: protandrous (males first) soldier production, unbiased soldier sex ratio, a very strong female bias in dispersers, and relatively low skew. Moreover, *K. morrisi* and *K. intermedius* exhibit sex ratio patterns intermediate between these two extremes.

These patterns suggest sex ratio manipulation by foundresses, constraining the reproductive strategies of soldiers. Thus, protogyny, as found in *K. habrus* and *K. waterhousei*, forces soldier females to produce male reproductives because they are unmated. The foundress is favored by frequency-dependence to produce most or all of the female reproductives, resulting in higher skew and a relatively unbiased disperser sex ratio. By contrast, protandry may be favored to ensure that female soldiers can sibmate soon after eclosion, under a breeding system with especially strong inbreeding (as in *K. hamiltoni*); in such a situation, a foundress can be more closely related to her granddaughters than her daughters (Kranz et al. 1999), which should also favor the evolution of relatively low reproductive skew.

Protandrous soldier-production may also engender higher motivation (and perhaps more-developed defensive morphology) in male soldiers than female soldiers, since after male soldiers have mated, their reproductive opportunities diminish and their main option for further maximizing inclusive fitness relies on alloparental defence. This prediction may help explain the unusual, unbiased solder sex ratio found in *K. hamiltoni*. This is not true for female soldiers, who can continue to oviposit for as long as their offspring will have time to develop. Because production of dispersers is limited by gall size, and not by the number

of female soldiers, foundresses may be able to encourage defensive behavior in their sons by overproducing males. By contrast, protogynous soldier production should instead involve relatively high levels of investment in defense by female soldiers, in part because higher reproductive skew means that female soldiers gain relatively more in fitness from alloparental behavior. Preliminary data from *K. habrus* supports such a tendency for female soldiers to defend the gall more readily than male soldiers (Crespi and Mound 1997).

Sex ratios patterns in social thrips are intimately associated with relatedness and inbreeding, but they appear not to be directly related, in extant species, to ecological and social-behavioral traits such as gall size, colony size, levels of skew, and efficacy of soldiers in defense (Crespi et al. 2004). These variables are, however, closely related among themselves, with gall volume positively correlated with number of soldiers and number of dispersers, gall volume and number of soldiers each negatively correlated with soldier efficacy in gall defense, and reproductive skew (here, the amount of reproduction by soldiers compared to foundresses) positively correlated with gall size and number of dispersers, and negatively correlated with soldier efficacy. The most parsimonious explanation for these findings is that gall size constrains the reproduction of soldiers, less soldier reproduction favors the evolution of better, more altruistic soldiers, and when soldiers are better fewer of them can be produced, leading to higher production of dispersers. Further analysis of the causes of interspecific and temporal sex ratio variation in thrips with soldiers requires fine-scale genetic and demographic studies, as well as data from additional species (e. g., the *K. waterhousei* species complex).

(4) SCENARIO FOR THE ORIGIN AND EVOLUTION OF SOLDIERS

Soldiers apparently originated soon after the host shift onto *Acacia* in the section Plurinerves from *Acacia* in the Juliflorae, after which two lineages, one with and one without soldiers, diversified onto the ancestors of *Ac. harpophylla*, *Ac. cambagei*, and the other microneurous Plurinerves species. In the lineage that developed soldiers, we have inferred that males commonly cofounded galls with females, relatedness and inbreeding levels were substantial within galls, and pressure from *Koptothrips* kleptoparasites was high. For unknown reasons, the galls induced by the thrips in this lineage were longer-lasting on the plants than those made by the lineages leading to *K. ellobus*, *K. acaciae*, *K. maslini* and *K. rugosus*. This increased gall persistence may have been related to a slower rate of nutrient input into the galls, leading to smaller galls, smaller broods, and an absence of physogastry. An extension of gall lifespan would have made galls vulnerable to invasion for a longer period, but it would also have made possible the evolution of two within-gall generations. At some point, the first-eclosing brood of the foundress would have had enough time to produce some offspring of their own within the gall.

The origin of soldiers centers on an evolutionary tradeoff between dispersal and staying, and for those who stay, options to reproduce, defend, or both. Staying within the natal gall would be favored by four main factors: (1) high costs to dispersal and attempting to found one's own gall, or (for males) mate with females after dispersal; (2) the ability of daughters of foundresses to increase fitness via personal reproduction or (for male protosoldiers) to mate with females in the gall; (3) the benefits of enhanced gall defense against *Koptothrips*; and (4) higher relatedness to disperser sibling gall-mates saved via defense than to one's own offspring that would be produced after dispersal. Along the lineage that evolved soldiers, these factors presumably tipped the balance towards adaptations of the first of the eclosing brood of a foundress to forego dispersal and specialize, behaviorally and morphologically, in within-gall reproduction and defense.

Costs of dispersal and independent gall induction, which represent the 'ecological constraints' posited as central to the evolution of alloparental care (Brown 1987; Emlen 1992; Koenig et al. 1992), are expected to be high in such arid-zone inhabiting insects.

However, we have no evidence as yet that such constraints were higher in the lineage leading to species with soldiers than in their sister-lineage that did not develop soldiers.

Genetic and dissection data have shows that female soldiers reproduce, sometimes to a substantial degree (Chapman et al. 2002). From these findings we have inferred a ability of female protosoldiers to reproduce, with especially-high reproductive rates in lineages nearer to the inferred origin of soldiers (Chapman et al. 2002). Moreover, we have observed male soldiers mating with sisters on numerous occasions, and male protosoldiers would have been the only mates available for protosoldier females. These findings suggest that personal reproduction by females and males was an important component of the inclusive fitness equation mediating the evolution of soldiers, especially because staying within the gall to help defend it may often have led directly to death from *Koptothrips*.

How much would colonies with protosoldiers have gained in enhanced gall defense? In the evolution of soldiers, the first individuals to eclose in their natal gall would have been morphologically and behaviorally similar to foundresses, which observations and collections show are capable of defending against *Koptothrips*. After protosoldiers became committed to remaining in the gall, there should have been strong selection for allocation of resources from dispersal adaptations to adaptations for reproduction, defense, or both. In other wing-polymorphic insects, wing-reduction in females engenders higher reproduction, earlier reproduction, or both (Harrison 1980; Roff 1986; Roff and Fairbairn 1991; Roff and Bradford 1996); moreover, male wing-reduction can involve earlier reproduction or higher allocation to weaponry (Crespi 1988). Each of these effects could have facilitated the rapid evolution of wing-reduction in female and male protosoldiers.

Enlarged forelegs would have been favored by selection for more effective defense against *Koptothrips*, which would benefit one's own offspring as well as offspring of the foundress and sisters. Behavioral experiments suggest that soldier propensity to defend is lower in more-basal species, such that defensive motivation and ability may be inversely related to levels of soldier reproduction. Thus, relatedness among soldiers is not high enough to remove all between-soldier conflict over reproduction and defense, and given potentially-high levels of personal reproduction, each soldier would prefer that a gall-mate took on the dangerous task of gall defense. Moreover, there are higher numbers of soldiers in more-basal species than in *K. habrus* and *K. intermedius*, so being less altruistic may incur lower costs in terms of successfully defending the gall.

Microsatellite data provides evidence that relatedness was not higher to gall-mates than to one's own offspring, at least in part because high levels of inbreeding tend to reduce such relatedness asymmetries (Chapman 2003). However, relatedness was apparently high between gallmates was high at the origin of soldiers (Chapman et al. 2002), and inbreeding levels F_{IS} of foundresses were also apparently substantial, on the order of 0.4-0.6. Such high inbreeding may also have made dispersing a more attractive option for incipient (still-winged) protosoldiers, if it engendered higher relatedness to their own offspring than to those of the foundress.

Their diversification of species with soldiers involved two main trends: (1) the evolution of higher levels of reproductive division of labor in the lineage leading to the sister-species *K. habrus* and *K. intermedius*, and (2) losses of the soldier caste in the lineages leading to *K. rodwayi* and *K. xiphius*. Stronger division of labor, involving higher skew and stronger defensive propensity, evolved in conjunction with relatively small gall size, low numbers of soldiers, and relatively low levels of the inbreeding coefficient F_{IS} of dispersers. The evolution of fewer soldiers should be favored when it results in a higher number of dispersers (especially given the sharply-limited brood capacity of the galls of K. habrus and K. intermedius). In turn, the presence of fewer soldiers may have selected for higher soldier motivation to defend, and higher soldier motivation should be favored when they

reproduce less and specialize more strongly in altruistic defense. The lower F_{IS} levels (on the order of 0.3) in *K. habrus* and *K. intermedius* may also have favored more highly-developed altruism, since under lower inbreeding levels, soldiers may be less related to their own offspring, and so may be more inclined to alloparental behavior (Chapman 2003).

The diversification of species with soldiers and their sister-group is also intimately associated with patterns of host-plant use. Thus, the sister group to the lineage with soldiers, ((*K. acaciae* + *K. ellobus*) + (*K. rugosus* + *K. maslini*)), exhibits the same pattern in host-plant relationships as the clade with soldiers: (1) a pair of sister species on *Ac. cambagei* (*K. hamiltoni*) and *Ac. harpophylla* (*K. harpophyllae*), and (2) use of a large number of closely-related 'microneurous' Plurinerves *Acacia* species (by the *K. rugosus* and *K. waterhousei* sibling species or host races), and (3) additional species being found in *Acacia* species (*e. g., Ac. orites, Ac. oswaldii, Ac. xiphophylla, Ac. melanoxylon,* and *Ac. calcicola*) that are notably distant from these (Crespi et al. 2004). Moreover, both losses of soldiers apparently occurred in association with host-plant shifts to phylogenetically-divergent *Acacia* hosts (Figure 2). In *K. rodwayi*, this shift led to habitation of mesic areas, which may reduced the strength of ecological constraint by making independent reproduction less costly (Kranz et al. 2002). In *K. xiphius*, the host-plant shift led to habitation of a highly arid region in the north of Western Australia, which may have engendered life cycle changes, such as shorter-lived galls, favoring solitary life.

All of the lineages that retained soldiers have remained on a suite of closely-related Acacia. except for *K. intermedius, which* appears to have undergone a major host-plant shift to *Ac. oswaldii*. Probably as a result of this shift, this species is highly unusual in several ways: (1) dispersers eclose within their natal gall, rather than leaving as second-instar larvae (as do all the other species with soldiers), (2) soldiers are partially winged rather than showing a near absence of wings, and wings are longer in soldiers that are smaller and have smaller forelegs (Crespi 1992a,b), (3) galls without any soldiers are not uncommon, which is never the case in other species, and (4) it exhibits a unique pattern of sex ratios, with strong female biases in both soldiers and dispersers (Table 1). Eclosion in the gall, wingedness of foundress offspring, lack of soldiers, and a female bias in dispersers are also present in the solitary species *K. rodwayi* (which also exhibits a very similar gall to that of *K. intermedius*) and these traits have apparently evolved in parallel, since these two species are not sister taxa.   These patterns suggest that *K. intermedius* and *K. rodwayi* been subject to similar selective pressures, leading to lower soldier numbers in *K. intermedius* and, in *K. rodwayi*, their loss altogether. However, *K. intermedius* also exhibits especially-high levels of reproductive skew between foundresses and soldiers, and a strong soldier defensive response. Might the benefits from producing increased numbers of dispersers sometimes outweigh benefits from defense by soldiers? In accordance with this hypothesis, *K. intermedius* exhibits the highest ratio of average soldier numbers (15) to average disperser numbers (60) of any species with soldiers.

(5) SIMILARITIES BETWEEN ACACIA THRIPS AND OTHER SOCIAL ANIMALS

Our studies have several broad implications for the study of animal social evolution. First, gall-inducing thrips support the 'factory-fortress' model for the evolution of eusociality (Alexander et al. 1991; Crespi 1994; Queller and Strassmann 1998). Thus, they exhibit high relatedness due in part to their claustral habitat, strong pressure from natural enemies due to the extremely high value of their nest resource, and the ability to defend against these enemies, using weaponry that originated in a different selective context. These findings support the hypothesis that there are two more or less discrete 'modes' of eusociality: 'factory fortress' species that include gall thrips, gall aphids, some termites, snapping shrimp, some mole rats, and a species of ambrosia beetle; by contrast, 'life-insurers' or 'forager-defenders', include most eusocial Hymenoptera and some social birds and mammals (Choe and Crespi 1997). Alloparental care and eusociality have apparently been favored by considerably different selective pressures across these two modes of

sociality, and broad-scale comparative studies on the causes of social evolution should consider them separately. Indeed, our findings suggest that the three main components of eusociality, behavioral, reproductive, and morphological divergence between more-reproductive and more-helpful individuals, evolve in a different sequence for factory-fortress social forms than for eusocial taxa with foraging workers (Chapman et al. 2002; Thorne et al. 2003). Thus, in gall-inducing *Acacia* thrips, morphological and behavioral differences between soldiers and dispersers apparently evolved before strong reproductive skew; by contrast, the usual scenario for Hymenoptera is that behavioral divergence evolves in conjunction with high skew, and that morphological divergence can evolve only after these two conditions have been met (Wilson 1971; Bourke and Franks 1995). We have hypothesized that the factory-fortress route to sociality differs because defense trades off less strongly with reproduction than does energetically demanding foraging, and because defensive morphology makes reproductive domination by foundresses less likely (Chapman et al. 2002).

Second, gall-inducing thrips with soldiers exhibit remarkable diversity in sex allocation ratio patterns, which are indicative of strong selection, and, we believe, parent-offspring conflict more or less resolved in different ways. We have suggested that foundresses use the timing and level of soldier and disperser sex ratio as a means to manipulate soldier alloparental and parental strategies towards their own interests. Coupled with our data on lack of relatedness asymmetry, these data suggest that one of the most important roles of haplodiploidy in social evolution is the ability to adaptively adjust sex ratios and produce males even when uninseminated.

Finally, our findings show that the selective pressures responsible for the origin and diversification of soldiers in Australian gall thrips on *Acacia* involve complex evolutionary dynamics that reach across three levels: (1) relations to host plants, (2) intraspecific interactions between gall morphology, reproductive skew, brood size, sex allocation, defensive behavior, morphology, relatedness, and inbreeding, and (3) pressure from natural enemies. Elucidating the links between and within these levels benefits from a 'model clades' approach to making social-behavior studies multidisciplinary in a phylogenetic framework, and it should repay the perseverant student of social evolution with new insights into how cooperation and altruism evolve among all organisms.

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