

High relatedness and inbreeding at the origin of eusociality in gall-inducing thrips

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Within the haplodiploid eusocial gall-inducing thrips, a species-level phylogeny combined with genetic data for five eusocial species enables an inference of levels of relatedness and inbreeding values for lineages at the origin of eusociality. Character optimization using data from five eusocial species indicates that the lineage or lineages where eusociality is inferred to have originated exhibit relatedness of 0.64–0.92, and F_{IS} of 0.33–0.64. The high inbreeding coefficients found in these eusocial thrips have increased relatedness among and within both sexes and have reduced the haplodiploidy-induced relatedness asymmetries [Hamilton, W. D. (1964) *J. Theor. Biol.* 7, 1–52]. These results indicate that unusually high relatedness is associated with the origin of eusociality, and they suggest a role for inbreeding in the evolution of bisexual helping.

Australian gall-inducing thrips gain food and shelter, in the form of a gall, from species of *Acacia* trees (1–6). The gall is formed as a female feeds on a developing phyllode (a petiole modified to serve as a stem and leaf) that encapsulates her and in some cases a male (4, 6). The foundress oviposits within the gall, and the developing thrips feed by sucking out the contents of plant cells on the gall's inner wall (1). In eusocial species, the first cohort to eclose are gall-bound soldiers, which are distinguished by robust forelimbs, reduced or absent wings, and self-sacrificing behavior exhibited in defense of the gall to the benefit of dispersing sisters and brothers (2, 4, 7). However, in at least one species, *Kladothrips hamiltoni*, it is suspected that soldiers could also be defending a few of their own offspring, as well as nieces and nephews, because soldiers are suspected of at least some egg laying within their natal gall (8). The foundress usually lives long enough to overlap with the adult soldiers, and she usually dies some time before the next group of individuals, the dispersive macropterae, reach the adult stage. Two life-history observations indicate that relatedness of individuals within a gall may be high: (i) multiple founding by females, which would reduce relatedness among brood, is not observed for any of the gall-inducing species on *Acacia* (3, 4), and (ii) sex ratios of dispersing brood are markedly female-biased (3, 9), which suggests the presence of strong local mate competition and inbreeding.

We developed microsatellite markers (10) for species of gall-inducing thrips with soldiers and used them to estimate genetic relatedness [refs. 11 and 12; RELATE 4.2c (<http://gsoft.smu.edu/GSoft.html>) by K. R. Goodnight and D. C. Queller] and inbreeding in five of the species with soldiers (Fig. 1; Table 1). In the four species for which multiple populations were sampled, the intraspecific similarity of relatedness and inbreeding estimates for populations that were up to 500 km apart indicates that these genetic parameters can be treated as species-specific values. These estimates of relatedness and inbreeding are among the highest ever recorded for social animals, and their magnitude is consistent with a high incidence of single-mating by foundresses, and brother-sister mating by both soldiers and dispersers (10). In addition, the strong relatedness asymmetries expected in outbred haplodiploid species (13) (e.g., a brood produced by a singly mated foundress is expected to exhibit

relatedness among sisters of 0.75 whereas relatedness of sisters to brothers is expected to be 0.5) are not at a detectable level in the four species in which they can be estimated, as expected given their high levels of inbreeding (14). For example, from Table 1, *K. hamiltoni* population 3 exhibits among-sister relatedness of 0.85 and sister to brother relatedness of 0.82 ($t = 1.95$, $P > 0.05$, $n = 12$).

Mapping of the relatedness and inbreeding estimates onto a phylogeny of Australian gall-inducing thrips on *Acacia* (15) is shown in Fig. 1. There is equal parsimony for the inference of one origin of the soldier caste (described in Fig. 1 legend as the first scenario) or two origins of the soldier caste (described in Fig. 1 legend as the second scenario), illustrated in Fig. 1 by a single trichotomy (15). Therefore, two separate analyses of ancestral states were conducted taking into account these plausible scenarios. By character optimization using squared-change parsimony (16), and the one origin of the soldier caste scenario, among-soldier relatedness on the branch in which soldiers are inferred to have originated is 0.78, and for females the inbreeding coefficient F_{IS} is 0.58. For the two-origin hypothesis, the inferred relatedness and F_{IS} estimates are even higher for the origin of soldiers for the sister taxa *K. hamiltoni* and *Kladothrips harpophyllae* (0.92 and 0.81, respectively, via character optimization in these two taxa considered separately), and the estimates remain high (0.64 and 0.33, respectively) for the origin of soldiers in *Oncothrips*. These results indicate that our inference of high relatedness and inbreeding at the origin or origins of eusociality in thrips is robust to phylogenetic uncertainty. Moreover, the possible presence of similarly high relatedness and inbreeding values in related noneusocial species would not refute the claim that these high values have played important roles in the origin of eusociality but would indicate instead that interspecific variation in genetic factors alone is not sufficient to explain the phylogenetic distribution of eusociality in this clade (6).

In the haplodiploid eusocial thrips, we have inferred high levels of relatedness for the origin or origins of eusociality, and these high values have been promoted by inbreeding, which in some species is quite strong. Inbreeding reduces the genetic relatedness asymmetries caused by haplodiploidy (14) that have been presumed to favor helping by females rather than males in Hymenoptera, and some models predict that it can promote the evolution of altruism in and of itself (17–22). High inbreeding levels in eusocial thrips are thus consistent with the presence in these species of soldiers of both sexes (4, 6). However, bisexual helping in thysanopteran soldiers may also be facilitated by the ability of males to mate within galls (23, 24), and by the close phenotypic similarity of males and females in this group (25), in

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Table 1. Within-colony relatedness in eusocial thrips

Species and population	Microsatellite data	Mother-to-daughter	Mother-to-son	Sisters	Brothers	Sister-to-brother	Brother-to-sister
<i>K. hamiltoni</i> : 1	19 galls, hamGATA1, 5 alleles	0.95 ± 0.03	1.00 ± 0.00	0.93 ± 0.04	0.95 ± 0.05	1.00 ± 0.00	0.89 ± 0.07
<i>K. hamiltoni</i> : 2	9 galls, hamGATA1, 4 alleles	0.76 ± 0.26	1.00 ± 0.00	0.83 ± 0.11	0.79 ± 0.14	0.76 ± 0.18	0.52 ± 0.23
<i>K. hamiltoni</i> : 3	12 galls, hamGATA1, 5 alleles	0.89 ± 0.03	1.00 ± 0.00	0.85 ± 0.04	0.86 ± 0.15	0.82 ± 0.18	0.62 ± 0.22
<i>K. harpophyllae</i> : 1	7 galls, hamGATA1, 2 alleles	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
<i>O. tepperi</i> : 1*	8 galls, TeppAAT1, 5 alleles; TeppAAT2, 3 alleles			0.76 ± 0.10		0.85 ± 0.15	0.64 ± 0.14
<i>O. tepperi</i> : 2	8 galls, TeppAAT1, 5 alleles; TeppAAT2, 2 alleles			0.82 ± 0.09	0.62 ± 0.08	0.81 ± 0.08	0.57 ± 0.15
<i>Oncothrips habrus</i> (melvillei): 1*	15 galls, TeppAAT1, 5 alleles; TeppAAT2, 3 alleles	0.66 ± 0.09	0.93 ± 0.05	0.61 ± 0.07	0.63 ± 0.09	0.59 ± 0.07	0.46 ± 0.07
<i>O. habrus</i> (melvillei): 2	7 galls, TeppAAT1, 9 alleles; TeppAAT2, 9 alleles	0.40 ± 0.08		0.65 ± 0.06			
<i>O. morrisi</i> : 1	8 galls, MorAAT1, 8 alleles	0.62 ± 0.12	0.94 ± 0.04	0.63 ± 0.11	0.52 ± 0.11	0.62 ± 0.12	0.38 ± 0.10
<i>O. morrisi</i> : 2	8 galls, MorAAT1, 8 alleles	0.43 ± 0.24	0.64 ± 0.36	0.72 ± 0.10	0.79 ± 0.21	0.74 ± 0.21	0.49 ± 0.15

Microsatellite development and primer sequences for loci TepAAT1 and TepAAT2 are reported elsewhere (10), and two additional primer pairs were utilized: HamGATA1 + 5'ACAAATCAGCATAAATTTTATA3' and - 5'CACATCGAGGGGTAGTTTTTG3', MorAAT1 + 5'TACTTTCCCATCTCCGTGCTG3' and - 5'ATCTTTTT-GCGTTTTTCATTT3'. Collection sites and dates are as follows: *K. hamiltoni* 1: 41 km south of Oodnadatta, South Australia, February 28, 1996; 2: 81.2 km east of Cadney Park, South Australia, April 24, 1997; 3: 47 km east of Birdsville, Queensland, April 7, 1998. *K. harpophyllae* 1: 34 km west of Charter's Towers, Queensland, April 3, 1998 (six galls monomorphic for one allele; one gall monomorphic for another allele); additional data on this rare species should increase the confidence with which we can conclude that they are truly clonal or nearly clonal. *O. tepperi* 1: 35 km south of Gilgandra, New South Wales, June 14, 1993; 2: Port Augusta, South Australia, June 18, 1996. *O. habrus* (melvillei) 1: 46 km southeast of Warren, New South Wales, June 14, 1993; 2: 2.3 km south of Ivanhoe, New South Wales, June 20, 1996; *O. morrisi* 1: 20 km east of Curtin Springs, Northern Territory, March 7, 1996; 2: 139 km west of Oodnadatta, South Australia, February 29, 1996. *Estimates were published elsewhere (10).

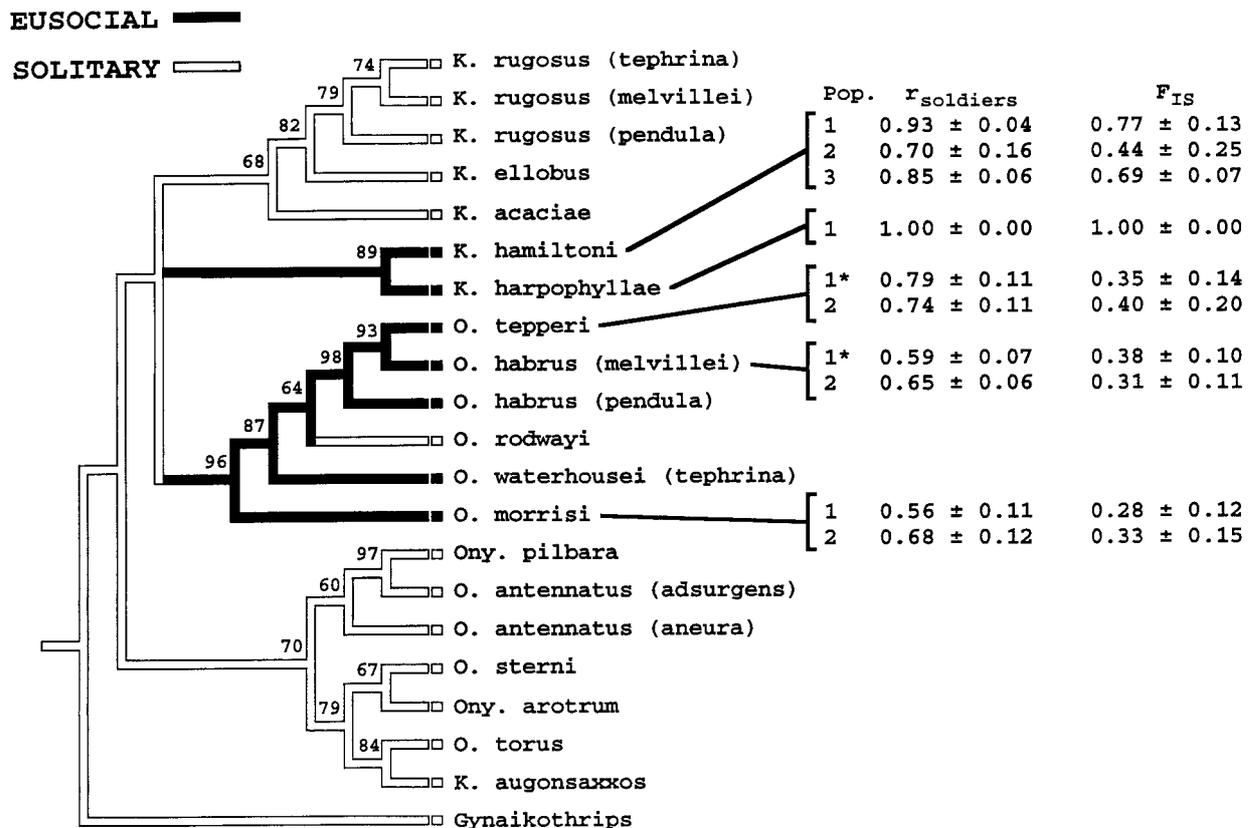


Fig. 1. Maximum-parsimony bootstrap 50% majority-rule consensus tree (plus compatible groups) of solitary and eusocial thrips, from Crespi *et al.* (15). Bootstrap values >50%, from 500 replicates, appear beside branches. Two plausible scenarios for the origin of the soldier caste are inferred: (i) one origin with the sister taxa *K. hamiltoni* and *K. harpophyllae* basal to all eusocial taxa or (ii) two origins with *Oncothrips morrisi* as the most basal of the eusocial *Oncothrips* and *K. hamiltoni* and *K. harpophyllae* represent a second independent origin of the soldier caste. Female soldier inbreeding and among-soldier relatedness values (for both sexes combined) are given, with associated jackknife standard errors (12). Bracketed names are species names for *Acacia* host plants (6, 14). Estimates for *Oncothrips tepperi* population 2 are for disperser females rather than soldiers. We have been collecting these thrips throughout Australia for 10 years and are confident that additional species with soldiers, basal to *K. hamiltoni* and *K. harpophyllae*, are unlikely to exist. The genus name *Onychothrips* is abbreviated as "Ony." An asterisk indicates that estimates were published elsewhere (10).

comparison to Hymenoptera, within which females appear to be phenotypically preadapted for helping (26, 27). Helping by both sexes is also associated with strong inbreeding in the diploid naked mole rats (28) and possibly some termites (29), and the apparent clonality or near-clonality of colonies of *K. hamiltoni* and *K. harpophyllae* demonstrates remarkable convergence with the clonal or nearly clonal colonies of gall-inducing aphid species with soldiers (30). Further studies of sex ratio selection, demography, sex biases in defensive behavior (4), and soldier repro-

duction in gall-inducing thrips should clarify the precise roles of relatedness, inbreeding, haplodiploidy, and ecological factors in the social evolution of Thysanoptera and other animals.

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