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Phylogenetics of gall-inducing thrips on Australian *Acacia*

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Analysis of DNA sequence data from four genes (*Elongation Factor-1α, wingless*, 16S rDNA and *cytochrome oxidase I*) yielded a well-resolved, well-supported phylogeny for all 21 species of gall-inducing thrips found on Australian *Acacia*. This phylogeny was then used to investigate the evolution of various behavioural and life history traits, and to examine the level of agreement with the taxonomy of the group. Our results suggest that there may have been a single origin of soldier castes in gall-inducing thrips. Examination of the distribution of the three primary life history strategies employed by these thrips (pupating in the gall, pupating in soil with soldier castes and pupating in soil without soldier castes) indicates that two of the strategies may have evolved as a result of factors associated with host plant affiliations or through parasite pressure. Our phylogeny does not support the existing generic classification of the group in that the genera are not monophyletic, nor does it lend itself to a clear solution to improve the classification in accordance with the phylogeny.

ADDITIONAL KEY WORDS: Thysanoptera – Phlaeothripidae – kleptoparasite – soldier castes.

INTRODUCTION

One useful approach to studying the evolution of behaviour and life history involves the reconstruction of phylogenies and the inference of ancestral states and evolutionary transitions (e.g. Martins, 1996). This method has been applied with increasing frequency to analysis of the evolution of social systems and their ecological and life-historical causes and consequences (e.g. Arnold & Owens, 1998, 1999; Faulkes *et al.*, 1997). Such analyses have begun to reveal convergences between disparate taxa (Choe & Crespi, 1997) and parallel patterns in the origins and losses of sociality (Wcislo & Danforth, 1997). However, additional phylogenetic studies of diverse taxa are required to develop a clear and full understanding of the macroevolution of social behaviour.

In recent years there has been an increasing level of interest in the species of Australian gall-inducing thrips found on *Acacia* (Chapman & Crespi, 1998; Crespi, Carmean & Chapman, 1997; Crespi *et al.*, 1998; Kranz *et al.*, 2000). One of the primary reasons for this interest has been the presence of five eusocial species among the 21 described species of gall-inducing *Acacia* thrips (Crespi, 1992b; Mound & Crespi, 1995; Mound, Crespi & Kranz, 1996). The evolution of eusociality among insects is a question that has fascinated evolutionary biologists for many years, and explaining the taxonomic distribution of the origins of eusociality continues to pose problems. The majority of studies examining the origins of eusociality have focussed on the order Hymenoptera (ants, bees and wasps). This has resulted in many of the hypotheses explaining the evolution of eusociality being readily applicable only to hymenopterans. One such hypothesis is the '3/4 relatedness hypothesis' (Hamilton, 1964; Trivers & Hare, 1976; Wilson, 1971) which proposes that the haplodiploid sexual system found in the order Hymenoptera will lead to high levels of relatedness among female kin which will, in turn, promote the evolution of eusociality. Pre-adaptations for sociality, such as the ability to continually return to a nest (Wcislo, 1992), and the ability to defend the

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nest (Starr, 1985, 1990) have also been implicated. More recently, research has moved away from the possible effect of haplodiploidy and has begun to concentrate on ecological constraints to independent nesting (e.g. Bull & Schwarz, 1996; Herbers, 1986) and benefits such as enhanced per capita brood production and enhanced defence against predators (e.g. Schwarz, 1988, 1994).

Up until the early 1990s, the Hymenoptera was the only group in which haplodiploidy seemed to coincide with multiple origins of eusociality. However, Crespi (1992b) showed that eusociality had also evolved in a gall-forming thrips lineage, and this has special importance for understanding social evolution because, like Hymenoptera, thrips are haplodiploid. Subsequent research on thrips phylogeny, ecology and intra-gall relatedness have suggested that both ecology and relatedness may be crucial factors underlying the evolution of sociality in this group (Chapman & Crespi, 1998; Crespi *et al.*, 1997, 1998; Crespi & Mound, 1997; Morris *et al.*, 1999). Crespi (1994) emphasized the importance of ecology in the evolution of sociality in group living species by suggesting that food-shelter coincidence, strong selection for defence and the ability to defend, should be adequate to facilitate the evolution of eusociality.

However, none of the factors that may facilitate the evolution of eusocial behaviour can be compared rigorously or identified without a robust phylogeny of the species in question. In such comparative studies, the significance of any life history, ecological or behavioural trait can only be assessed when the phylogenetic structure of the group can be accounted for (Felsenstein, 1985b). Thus, a robust phylogeny is essential in order to determine the origins of traits and the degree to which the evolutionary relationships of a group are related to the attributes of its extant species.

Previous studies of the phylogenetic relationships of the *Acacia* gall-inducing thrips have been only partially successful in that, while phylogenies have been produced, they have tended to lack resolution and have omitted a number of taxa (Crespi *et al.*, 1998; Morris *et al.*, 1999). The primary aim of this study therefore, is to reconstruct a phylogeny for all 21 described species of gall-inducing thrips from *Acacia*. We then examine the origins of some life history and behavioural traits and discuss the implications of these for future studies of these remarkable thrips. One such life history trait to be examined is the presence of a 'soldier morph' that is indicative of eusociality in these species. The evolution of subfertile 'soldiers' in some gall thrips may be due, in part, to a trade-off between producing one's own offspring and defending relatives within the gall, and thus maximizing inclusive fitness by enhancing the production and survival of dispersing adults produced by the foundress and other relatives (Crespi, 1992b). In order to examine the evolution of soldier morphs this trait will be mapped onto the gall thrips phylogeny and thereby allow the origins and losses of the trait to be identified.

Another aim of our study is to examine the extent to which the phylogeny of gall thrips is in agreement with the existing generic classification of these species. Previous hypotheses of the gall thrips phylogeny have been at odds with the classification, and indicate that some, or all, of the three current genera may be paraphyletic. Thus we will test the monophyly of each of the three gall-inducing genera, using our phylogeny and discuss the implications for future taxonomic work on the group.

GALL THRIPS LIFE HISTORY AND BEHAVIOUR

The majority of *Acacia* species in Australia conduct photosynthesis through phyllodes rather than the bipinnate leaves typical of *Acacia* species found in other parts of the world. Phyllodes are leaf-like structures formed from modified petiole tissues, and are assumed to be an adaptation to the arid conditions experienced over much of Australia. Some species of thrips cause galls to form on *Acacia* phyllodes as a result of feeding activity (rather than through oviposition, as is the case with some other insects such as chalcidoid wasps). The feeding by thrips causes a phyllode to develop into an expanded pouch or to curl or roll so that the opposing edges of the phyllode meet or overlap to form a more or less sealed chamber. The adult thrips is thus enclosed within this chamber of plant tissues and proceeds to lay eggs on the inner surface of the gall (Mound, 1994).

Galls are most often induced by single adult females but in some species galls may be established by a male–female pair (Crespi, 1992a; Kranz *et al.*, 2000). The eggs hatch after a period of days (varying with species) into larvae and, from this point, follow one of three paths depending on the species to which they belong. The first path is for these larvae to develop into pupae and then become fully winged adults that leave the gall when conditions are favourable, and subsequently initiate galls in which to produce their own offspring. The second type of life history seen in gall-inducing thrips is one where the larvae leave the gall prior to becoming pupae. To date, it is not clear how the larvae leave the gall. It may be that the host plant sheds the galled phyllodes and the gall then dries out and opens up, or that ageing of the phyllode while it is still attached to the tree causes the gall to open and the larvae then emerge. It has been suggested that these larvae pupate in the soil and emerge as adults to create new galls when climatic conditions promote new growth of the host *Acacia* species (Crespi & Abbot,

1999). This life history strategy appears to maximize brood size and minimize the time spent in development within the gall. In the third type of life history, the first generation of eggs develop into individuals that exhibit 'soldier' morphology; adults with reduced wings and antennae, but enlarged forelimbs, whose role appears to be one of defence. The second cohort of eggs in these species also leave the gall as larvae to pupate elsewhere (except for *Oncothrips tepperi* where individuals disperse as winged adults), and then develop into fully winged, fully reproductive adults who presumably begin the cycle of gall initiation again. This second group of eggs is primarily produced by the original foundress in many of the species that have 'soldiers', but in a few species a large proportion of the dispersing generation can be produced by the soldiers or 'gall morphs' (e.g. *O. morrisi*). It is species that have this last type of life history that can be considered to be eusocial, as the soldier caste is often significantly less fecund than the foundress (Chapman *et al.*, 2000). This life history strategy results in a situation where the thrips species have extended parental care (through the soldiers' defence and maintenance of the gall), overlap of generations, and a semi-sterile caste (*sensu* Wilson, 1971). One exception to this life history strategy is that seen in *Oncothrips sterni*. In *O. sterni* the first generation of offspring develop into an apterous 'gall morph' that has not been observed to defend the gall. It has been suggested that this apterous non-soldier morph may represent a precursor to the soldier morph seen in other species of *Oncothrips* and *Kladothrips* (Mound *et al.*, 1996). The apterous gall morphs presumably contribute significantly to next generation of dispersing, macropterous individuals that establish new galls in turn.

MONOPHYLY OF THE GALL-INDUCING THRIPS

One aspect of gall-inducing thrips phylogenetics that has not yet been established is whether the 21 described species found on *Acacia* form a monophyletic group. On current morphological evidence, the species of thrips found on *Acacia* are believed to constitute a monophyletic group (Mound, 1971; Mound *et al.*, 1996). Thus far, there is no evidence to suggest that any other thrips species from Acacia falls within the clade formed by the gall-inducing species discussed here (Morris, 2000). However, given that the Australian thrips fauna is largely unknown, it is difficult to make assumptions about what selection of taxa might allow this hypothesis to be rigorously tested. As such we continue to assume that the gall-inducing thrips species found on *Acacia* are a monophyletic group until evidence to the contrary becomes available.

MATERIAL AND METHODS

TAXON SAMPLING

Our analyses included all 21 of the described species of gall-inducing thrips found living on *Acacia* in Australia. These species are divided into three genera (*Kladothrips*, *Oncothrips* and *Onychothrips*), on the basis of morphological characters (Mound, 1971; Mound & Crespi, 1995; Mound *et al.*, 1996). In addition to the gall-inducing species, *Rhopalothripoides froggatti* and two representatives of the genus *Dactylothrips* were included as outgroup taxa. These outgroups were chosen because they were the most closely related taxa to the gall-inducing genera in molecular analyses of thrips on *Acacia* (Morris, 2000), and are also believed to be close relatives based on morphology (Mound, 1971). A list of the taxa used in this study, together with collection site details and host plant affiliations can be found in Table 1. Voucher specimens of all taxa used have been deposited in the Australian National Insect Collection (ANIC) at CSIRO Entomology, Canberra.

TISSUES AND DNA EXTRACTIONS

DNA for this work was extracted from fresh, frozen, and ethanol-preserved material. In most cases multiple individuals were pooled to obtain adequate quantities of DNA. Thrips to be pooled for extractions were always taken from the same gall, and therefore can be assumed to be genetically highly similar. DNA was extracted from fresh material using a phenol/chloroform protocol as described in Crespi *et al.* (1996). For frozen and ethanol-preserved material DNA was extracted using Chelex 100 resin (Walsh, Metzger & Higuchi, 1991). Specimens for Chelex extraction were homogenized in 25 µl of Tris buffer (pH 8.0) and then 100 µl of 5% Chelex 100 resin was added prior to a 5-h incubation period at 55°C. After incubation, the sample was vortexed and then spun down in a centrifuge for approx 5 s (at 10 000 *g*) before incubation for 10 min at 95°C. The sample was then vortexed again and centrifuged for 30 s at 10 000 *g* before use in PCR amplifications.

POLYMERASE CHAIN REACTION (PCR) AMPLIFICATION AND SEQUENCING

PCR amplifications were performed using the following protocol: 94°C, 1 min denaturation; 48–52°C, 45 s annealing; 72°C, 1 min extension for 35 cycles; with a final cycle of 5 min extension at 72°C. The polymerase enzyme used was Amplitaq Gold (Perkin Elmer) which required a 9 min 95°C incubation step for the first cycle only, as instructed by the manufacturer. PCR reaction mixtures consisted of 50 µl of 1 × reaction buffer (Amplitaq Gold), containing 4 mM $MgCl_2$, 0.8 mM dNTPs, 10 pmol of each primer, approx 50 ng

Table 1. Host plant and collection localities for the species of gall-inducing thrips and outgroups used in this study

Species	Host *Acacia*	Location
Kladothrips acaciae	*A. harpophylla*	Mungindi, Qld. April 1997
Kladothrips augonsaxxos	*A. aprepta*	Wallumbillah, Qld. April 1997
Kladothrips ellobus	*A. cambagei*	Emmet, Qld. April 1998
Kladothrips hamiltoni	*A. cambagei*	Coober Pedy, SA. January 1999
Kladothrips harpophyllae	*A. harpophylla*	Moonie, Qld. April 1997
Kladothrips maslini	*A. orites*	Lismore, NSW. July 1996
Kladothrips rugosus	*A. maranoaensis*	Mitchell, Qld. March 1998
Kladothrips xiphius	*A. xiphophylla*	Mt Tom Price, WA. April 1997
Oncothrips antennatus	*A. aneura*	Meekatharra, WA. April 1998
Oncothrips habrus	*A. melvillei*	Mildura, NSW. June 1999
Oncothrips morrisi	*A. calcicola*	Coober Pedy, SA. January 1999
Oncothrips rodwayi	*A. melanoxylon*	Portland, Vic. October 1999
Oncothrips schwarzi	*A. kempeana*	Kulgera, NT. January 1999
Oncothrips sterni	*A. aneura*	Wittenoom, WA. July 1993
Oncothrips tepperi	*A. oswaldii*	Whyalla, SA. April 1999
Oncothrips torus	*A. citrinoviridis*	Meekatharra, WA. April 1998
Oncothrips waterhousei	*A. papyrocarpa*	Whyalla, SA. January 1999
Onychothrips arotrum	*A. aneura*	Kulgera, NT. January 1999
Onychothrips pilbara	*A. citrinoviridis*	Hamersley Gorge, WA. July 1993
Onychothrips tepperi	*A. aneura*	Quilpie, Qld. April 1998
Onychothrips zygus	*A. pickardii*	Maree, SA. April 1998
Rhopalothripiodes froggatti	*A. melanoxylon*	Canberra, ACT. August 1999
Dactylothrips sp.	*A. stowardii*	Quilpie, Qld. April 1998
Dactylothrips sp.	*A. cambagei*	Coober Pedy, SA. October 1999

Table 2. Primers used for PCR amplifications and sequencing of thrips in this study

Genes	Forward primers	Reverse primers
EF-1α	M51.9 (Cho *et al.*, 1995)	G346 (designed by D. Morris)
	5′-CAR GAC GTA TAC AAA ATC GG-3′	5′-AGA CTC AAC ACA CAT AGG TTT GGA C-3′
wingless	LepWG1 (Brower & DeSalle, 1998)	G348 (designed by D. Morris)
	5′-GAR TGY AAR TGY CAY GGY ATG TCT GG-3′	5′-GTT CGG TAT CCG CGT CCA CA-3′
16S	LR-J-12887 (Simon *et al.*, 1994)	LR-N-13398 (Simon *et al.*, 1994)
	5′-CGG GTC TGA ACT CAG ATC ACG T-3′	5′-CGC CTG TTT AAC AAA AAC AT-3′
COI	C1-J-2183 (Simon *et al.*, 1994)	A2735 (designed by B. Crespi)
	5′-CAA CAT TTA TTT TGA TTT TTT GG-3′	5′-AAA AAT GTT GAG GGA AAA ATG TTA-3′
	C1-J-2195 (Simon *et al.*, 1994)	
	5′-TTG ATT TTT TGG TCA TCC AGA AGT-3′	

of template DNA, and 1 unit of Amplitaq Gold polymerase. PCR products were purified using BRESASpin PCR purification columns (Geneworks) and then cycle sequenced using a Big-dye Ready-Reaction kit (Perkin Elmer) and the methods recommended by the manufacturer. Electrophoresis of sequence products was performed on an ABI 373 automated sequencer through the Institute of Medical and Veterinary Science, Adelaide.

Details of primers used to amplify (and sequence) each of four gene regions are given in Table 2. The four genes used in this study were *elongation factor-1α* (EF-1α), *wingless,* 16S rDNA (16S), and *cytochrome oxidase I* (COI). We were able to obtain sequences for 23 species of the required 24 for EF-1α, COI and 16S. However, we had difficulty amplifying some taxa for *wingless* and so the corresponding sequences were coded as missing data in our analyses. Those species that were not sequenced were; *K. harpophyllae* for COI, *O. torus* for EF-1α and 16S, and *K. harpophyllae, Oncothrips schwarzi, O. sterni, O. waterhousei* and *Onychothrips arotrum* for *wingless*.

The sequences obtained include, when aligned, 422 bp of coding sequence and an intron of about 100 bp for EF-1α, 445 bp for *wingless*, and 550 bp for COI. The 16S sequences include two regions (presumably corresponding to the loops in the secondary structure) where the alignments were ambiguous and as a result were excluded from our analyses. The 16S sequences used in our analyses were 386 bp in length after approximately 80 bp was removed. All sequences were aligned using Clustal X version 1.8 (Thompson *et al.*, 1997) and then imported into PAUP* (version 4.0b4a) (Swofford, 1999) for analysis. Sequences of the four genes used in our analyses can be found on Genbank under the accession numbers AF386650–AF386738.

PHYLOGENETIC ANALYSES

Each data set was first analysed independently with maximum parsimony methods. Parsimony analyses were conducted on unweighted data using the heuristic search option with tree bisection-reconstruction (TBR) branch swapping and random addition of taxa (100 replicates per search with ten trees held at each step). Each of the individual data sets were then subjected to statistical testing to determine the most appropriate model of evolution for use in maximum likelihood analyses, using Modeltest 3.0 (Posada & Crandall, 1998). Each data set was then analysed using the maximum likelihood optimality criterion employing the model of evolution proposed by Modeltest. Homogeneity of base frequencies was tested using the chi-squared test in PAUP*. Each data set was tested for potential saturation of the data by plotting unmodified distances against Kimura 2-parameter modified distances for first, second and third codon positions as well as for transitions and transversions.

The individual data sets were combined into a single data set of 1870 bp with each gene treated as a separate partition. This data set was then tested for congruence among the four genes using the partition homogeneity (incongruence length difference) test (Farris *et al.*, 1995), as implemented in PAUP*. The combined data were subjected to parsimony and maximum likelihood searches in the same manner as the individual data sets. Confidence measures for the nodes in the resulting trees were estimated using the bootstrap approach (Felsenstein, 1985a). Bootstrap values were determined using 1000 heuristic search replicates for parsimony analyses and 100 replicates for maximum likelihood analyses.

HYPOTHESIS TESTING

In order to test hypotheses about the evolution of behavioural traits among gall-inducing thrips we utilized the Templeton (Templeton, 1983) test as implemented in PAUP*. The Templeton test applies a Wilcoxon Signed Ranks test to the trees in question, in effect comparing the most parsimonious trees resulting from an unconstrained analysis with those obtained when one group is constrained to be monophyletic. We used the Templeton test for assessing our hypotheses because it is a non-parametric test and, as such, does not assume a normal distribution for the characters being tested (Crandall *et al.*, 1999).

To examine the distribution of character traits among the species of gall-inducing thrips, traits were mapped onto a phylogeny using MacClade 3.07 (Maddison & Maddison, 1997). Character evolution was assessed using the Trace Character option with the default 'most parsimonious state shown at each node' setting.

RESULTS

SEPARATE ANALYSES

DNA sequences for EF-1α, *wingless*, 16S and COI were obtained from 24 thrips species from sites around Australia. The nuclear genes used had relatively few parsimony informative sites (EF-1α = 56 out of 525, *wingless* = 53 out of 445), and showed low levels of divergence between taxa (0–4% and 0–6% respectively, based on Kimura 2 parameter distances). As might be expected, the mitochondrial genes used exhibited more parsimony informative sites (16S = 94 out of 368, COI = 189 out of 555), and higher levels of divergence (4–16% and 8–20% respectively). The parsimony analysis of the EF-1α and *wingless* data sets produced 216 and 12 most parsimonious trees respectively. Maximum parsimony analysis of the 16S and COI data sets each produced 6 equally most parsimonious trees. Analysis of these individual data sets using Modeltest 3.0 suggested that the most appropriate model of evolution for analysing the EF-1α data set was the TIM (Rodríguez *et al.*, 1990) with a gamma-shape parameter (Γ), and the model selected for the *wingless* data was TrNef + Γ (Tamura & Nei, 1993). The suggested model of evolution for the COI data set was the General Time Reversible (GTR) with gamma-shape and invariable sites (GTR + Γ + I), and the optimal model for the 16S data was TVM + Γ (Rodríguez *et al.*, 1990). The trees resulting from these maximum likelihood analyses are shown in Figure 1.

Analysis of potential saturation (based on the distance plots described above) of each data set suggested that saturation was unlikely to cause significant problems with our analyses. However, it should be noted that while base frequencies were not significantly different ($P = 0.35$) among different taxa in the data set, there was a distinct AT richness in the mitochondrial data sets. The 16S data consisted of approximately 80% A + T, and the COI data had an AT bias of 73% (65% for first and second codons and 88% for third

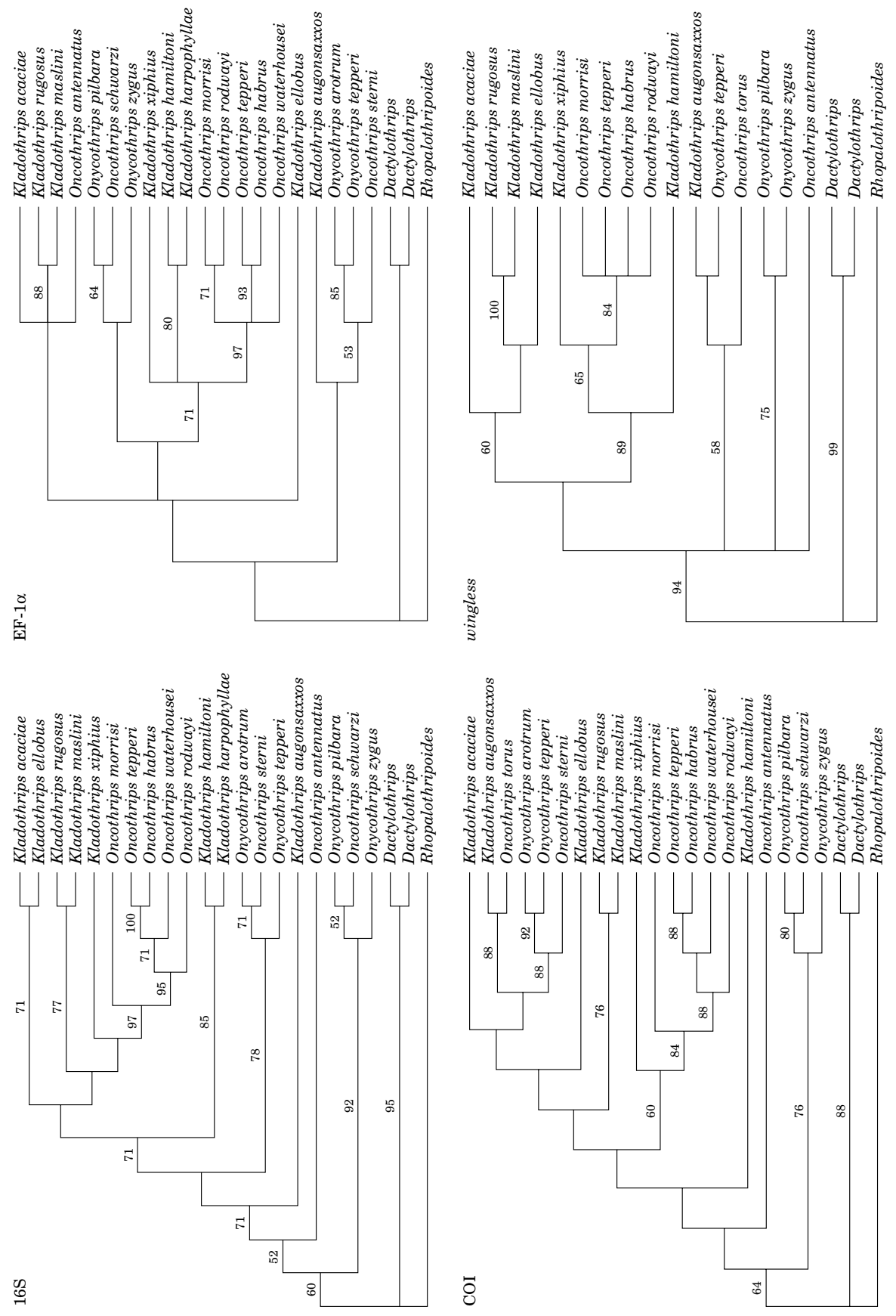

Figure 1. Trees resulting from maximum likelihood analysis of EF-1α, *wingless*, 16S, and COI data sets, with bootstrap values (100 replicates) shown above each branch.

positions). The high AT bias in COI third codon positions suggests that, while there is little evidence from the plots for saturation, it may well be occurring in effect because the probability of a substitution for a G or C is very low compared to substitutions between A and T.

For three of the separate data sets, the tree topology resulting from maximum likelihood analyses was very similar to that produced by parsimony analysis of the same data set, and for this reason the parsimony trees are not shown here. Only for the COI data set were the maximum likelihood and parsimony trees markedly different, and we attribute this to noise in the data set resulting from the A+T bias at third codon positions. There are some clades that are supported in all of the analyses for each data set (except where the sequence for a species was not available). The group ((*O. schwarzi*, *Ony. pilbara*), *Ony. zygus*) is found in all trees, as is a sister taxa relationship between *K. rugosus* and *K. maslini*. Another group recovered by all analyses is the clade containing *K. xiphius*, *O. morrisi*, *O. rodwayi*, *O. waterhousei*, *O. tepperi* and *O. habrus*. Within this group, relationships vary between data sets, but all analyses support *O. habrus* and *O. tepperi* as sister taxa. The clade containing *O. sterni*, *Ony. tepperi* and *Ony. arotrum* is not supported in all analyses as two of these taxa are missing from the *wingless* data set, but it is found in the remaining three data sets.

COMBINED ANALYSES

Once the four data sets were combined we tested them for incongruence using the partition homogeneity or incongruence length difference test (ILD) (Farris *et al.*, 1995). The result of this test on the combined data sets suggested that they were not significantly incongruent and thus could be combined ($P=0.79$).

The combined data set was then analysed using maximum parsimony and the single tree resulting from this analysis is shown in Figure 2. The evolutionary model selected as most appropriate for maximum likelihood analysis of these data was the general time reversible with gamma-shape and invariant sites (GTR+Γ+I). The phylogram resulting from a GTR+Γ+I heuristic search of the combined data sets is shown in Figure 2. The trees resulting from parsimony and maximum likelihood analyses are topologically identical.

HYPOTHESIS TESTING

We used Templeton tests to compare our most parsimonious tree with the shortest trees resulting from analyses where specific groups were constrained to be monophyletic. This test determines the probability that the constraint tree is significantly different from the most parsimonious tree resulting from an unconstrained search. The first hypothesis tested was that the thrips species exhibiting soldier morphs are a monophyletic group. This hypothesis is rejected using the Templeton test ($P=0.0001$), suggesting that there may be multiple origins of soldier morphs. Each of the genera of gall-inducing thrips were tested for monophyly and in all cases the Templeton test rejected the null hypothesis of monophyly (*Kladothrips*, $P=0.0030$; *Oncothrips*, $P=<0.0001$; *Onychothrips*, $P=<0.0001$).

DISCUSSION

The phylogeny presented here, inferred from four genes, is substantially better resolved and better supported than the one presented by Crespi *et al.* (1998), which relied on two mitochondrial genes and two small morphological data sets. As a result, this phylogeny provides a firm basis for evaluation of the monophyly and taxonomy of this group, reconstruction of ancestral behavioural and life history states, and inference of evolutionary transitions.

MONOPHYLY OF 'SOLDIER' THRIPS

There are seven species of gall-inducing thrips on *Acacia* that are known to exhibit a 'gall morph', or gall-bound caste that generally has reduced wings, truncated antennae and a neotenic appearance relative to that seen in the fully winged dispersing morph. In one of these species, *O. sterni*, the gall morph is wingless and has a very neotenic appearance and has not been seen to display any tendency toward fighting or defensive behaviour (Mound *et al.*, 1996). The gall morph of *O. sterni* is believed to be an adaptation to producing larger numbers of the dispersing generation. As such it might be expected that the wing reduced morph of *O. sterni*, rather than having reduced fecundity as is seen in the 'soldier morphs', would have increased fecundity. As *O. sterni* is not considered to have a true soldier morph, and because behavioural observations on this species are very limited, we will not discuss this species further.

The remaining six species with gall morphs have a more typical micropterous 'soldier' morph where, in addition to the reduction of the wings and antennae, there is a marked increase in the size of the fore femora and pronotum. The increase in these body dimensions is believed to be correlated directly with increases in muscle mass in these areas, to provide greater strength to the forelimbs, the primary weapons of these small insects.

All of the gall-inducing species with soldier morphs have a single common ancestor in our phylogeny, which would suggest that there may be a single origin of this life history trait. However, among the species

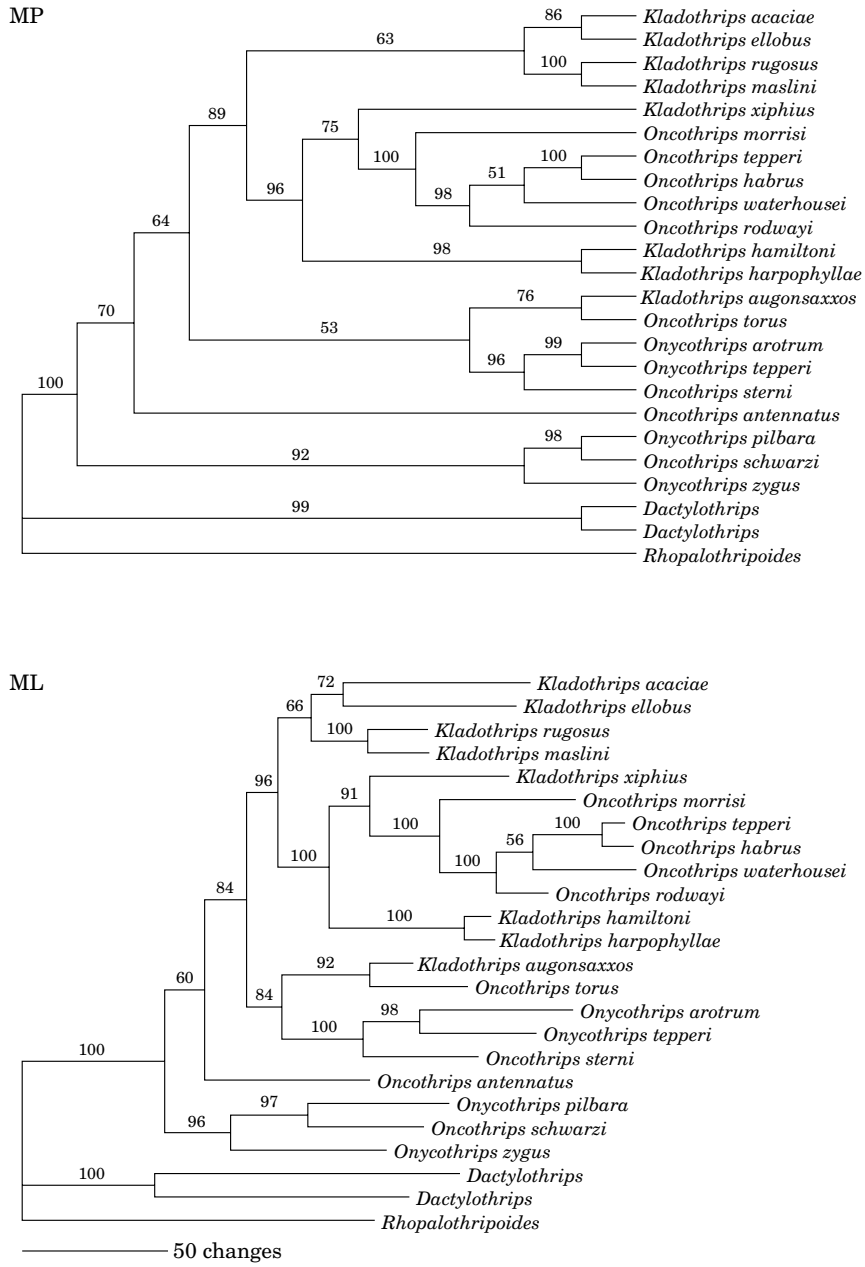

Figure 2. Trees resulting from maximum parsimony analysis (MP) and maximum likelihood analysis (ML) of combined data set (EF-1α + *wingless* + 16S + COI). (MP) Most parsimonious tree with bootstrap values (1000 replicates) shown. (ML) Maximum likelihood tree with branch lengths drawn proportional to the expected amount of character change and with bootstrap values (100 replicates) given above the branches.

descended from this ancestor are two that may not fit this pattern, *K. xiphius* and *O. rodwayi*. Neither of these species is known to produce soldiers or any form of gall morph.

On the basis of the tree resulting from our analysis there are two viable hypotheses regarding the number of origins of soldier morphs. The first hypothesis is that there is a single origin of the trait with two subsequent losses in *K. xiphius* and *O. rodwayi* (Fig. 3a). The second hypothesis is that there have been two origins of soldier morphs, one in the clade ((((*O. habrus*, *O. tepperi*) *O. waterhousei*) *O. rodwayi*) *O. morrisi*) with one loss in *O. rodwayi*, and a second origin in the clade (*K. hamiltoni*, *K. harpophyllae*) (see Fig. 3B). These two hypotheses remain equally valid, requiring the same number of evolutionary origins or losses of the trait of soldier castes (one origin and two losses versus two origins and one loss). There is little other

evidence to support one hypothesis rather than the other. It can be argued that losses of a complex trait might be more likely than origins, which would favour the first hypothesis. However, the presence of the non-soldier, non-dispersing morph in *O. sterni* suggests that in gall thrips, there may be a tendency towards the evolution of gall-bound morphs. Furthermore, the thrips species found in galls on *Casuarina* trees in Australia all exhibit wing-reduced gall morphs, suggesting that evolution of gall-bound morphs may be linked to a gall dwelling habit under arid conditions. This provides some support for the hypothesis that there are multiple origins of soldiers in the gall thrips.

A third possible hypothesis would also require three evolutionary steps to produce a similar distribution of the trait. In this hypothesis there would have to be three origins of soldier castes, one in (*K. hamiltoni*, *K. harpophyllae*), one in *O. morrisi*, and one in ((*O. tepperi*, *O. habrus*) *O. waterhousei*). This hypothesis would require no losses of the trait, but is not considered a highly plausible scenario. The reason that this last hypothesis is not supported is because it is assumed that *O. rodwayi* is an example of a species that has lost the trait, rather than one that has never possessed the trait. This is based on the fact that *O. rodwayi* foundresses produce brood sizes that are roughly the same as those seen in *O. waterhousei*, *O. habrus* and *O. tepperi*, rather than the much larger broods found in all of the species that do not have soldiers (Kranz, 2000). In addition, *O. rodwayi* is the only gall-inducing species that has a temperate rather than arid distribution, suggesting that loss of soldiers may have been facilitated by a relaxation of environmental or ecological selection pressures.

Our findings here exhibit an interesting parallel to those in other social insects, such as bees, aphids, social spiders and cooperatively-breeding birds, which are also characterized by evolution of sociality in a clade being followed by reversions to solitary behaviour (Danforth, Sauquet & Packer, 1999; Wcislo & Danforth, 1997). In gall thrips, we have inferred either one or two losses of eusociality and one of these, in the lineage leading to *Oncothrips rodwayi*, is associated with a host–plant shift to a more mesic environment. Moreover, our phylogenetic analysis suggests that species with soldiers evolved from a plesiotypic state of offspring reaching adult eclosion within their natal gall (see below); this evolutionary transition makes sense because it provides a clear selective context for soldier origin, through gall defence by early eclosing adults. Further analysis of the number and phylogenetic positions of origins and losses of sociality in thrips will require determining whether the soldier morphs in *Oncothrips* species are homologous to or convergent with those in *Kladothrips*, via detailed studies of caste allometry or unusual soldier traits shared between lineages.

DISTRIBUTION AND EVOLUTION OF LIFE HISTORY STRATEGIES

There are three broad life history strategies seen in gall-inducing thrips on *Acacia*. The first of these strategies is one where the thrips initiate galls on growing phyllodes, lay eggs, and these eggs then develop from larvae to pupae and become adults within the gall. The adults then wait within the gall for an appropriate time to disperse (presumably after rain has stimulated new growth on the host *Acacia*) and begin initiating galls for their own brood. This strategy appears to be the plesiotypic state, as all of the basal lineages in the phylogeny of gall-inducing thrips lead to species that utilise such a strategy (see Fig. 4). The two more derived species that also possess a similar life history are *K. maslini* and *O. rodwayi*. Both species are unusual among gall thrips in that they are the only species found in temperate climates. Thus it might be said that this plesiotypic life history strategy is the default setting, to which *K. maslini* and *O. rodwayi* have 'reverted'.

The other two life history strategies are seen as alternative answers to the problem of avoiding parasitization and maximizing offspring survivorship. The first of these derived strategies is to produce a large number of offspring that leave the gall as second-instar larvae. The second derived strategy is one in which a smaller number of brood are produced, some of which have soldier morphology in order to defend the remaining offspring. The species that employ either of these two derived strategies have many factors in common, and as such it is difficult to assess precisely which of these common factors may have promoted the evolution of the two derived life history strategies. Two of the most obvious factors that the species displaying a derived life history strategy have in common is that they are all found on host plants from the Section Plurinerves of *Acacia*, and that they all are attacked by species of the kleptoparasitic genus *Koptothrips*.

It is possible that the use of certain host plants may have influenced the evolution of new life history strategies in gall-inducing thrips. With the exception of *K. maslini* and *O. rodwayi*, all the thrips species with the plesiotypic strategy are found on host plants of the Section Juliflorae (see Fig. 5). However, all the remaining species with the alternative derived strategies are found on hosts from Section Plurinerves, a demarcation that may have some significance. The one species with a derived life history strategy that is found on a Juliflorae host plant is *K. xiphius* but, in this case, the plant classification may be in error as

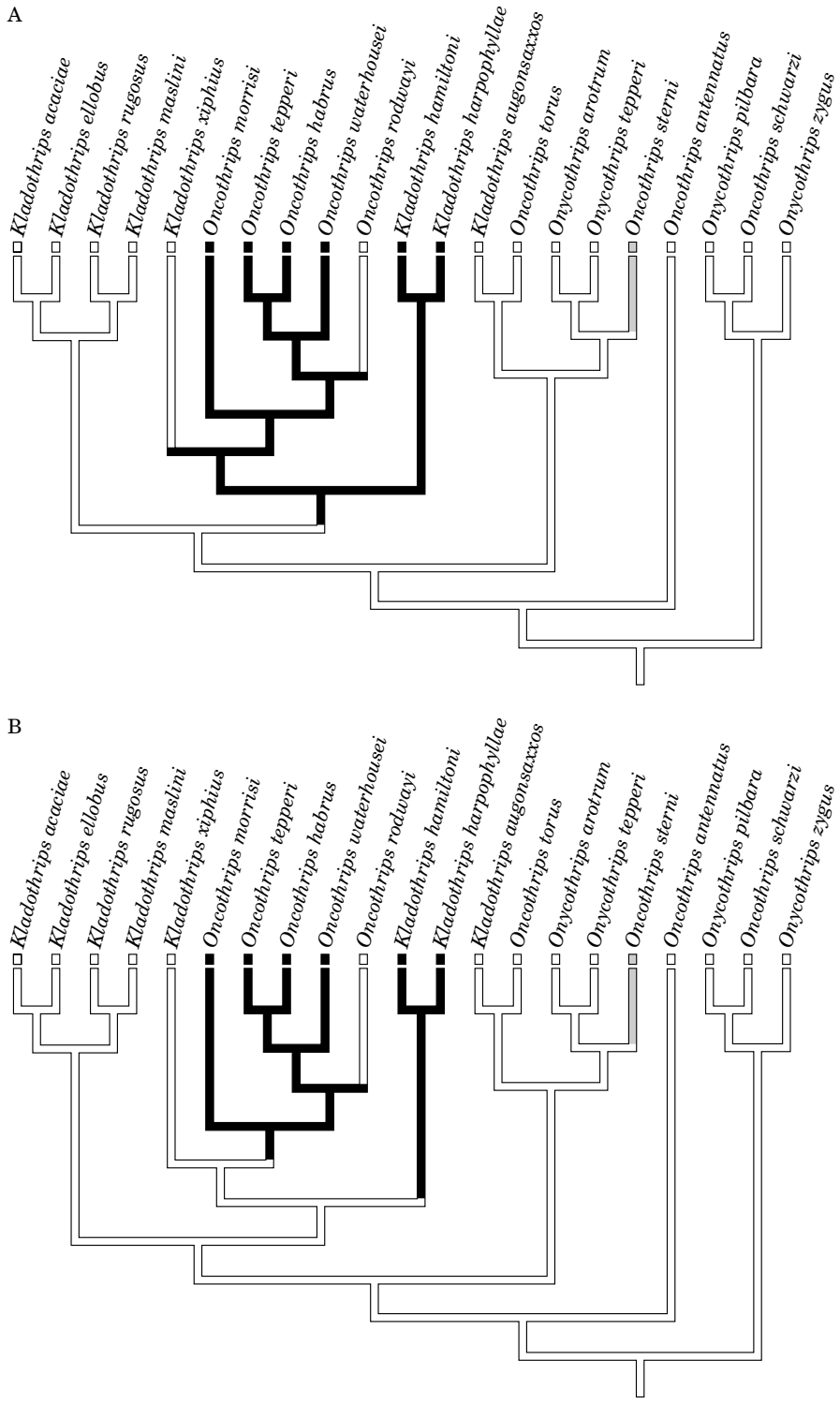

Figure 3. A, most parsimonious tree with a hypothesised single origin of soldier castes mapped. B, most parsimonious tree showing a hypothesis of two origins of soldier castes mapped. Black bars denote species with soldier castes, grey bar indicates species with non-soldier gall morph and white bars indicate species that do not have soldier castes.

the closest relative of *Acacia xiphophylla* (the host of *K. xiphius*) is found within the Section Plurinerves not Juliflorae (B. Maslin, pers. comm.)

The other factor that may have led to the evolution of the derived life history strategies is the presence of *Koptothrips*. *Koptothrips* is a genus of kleptoparasitic

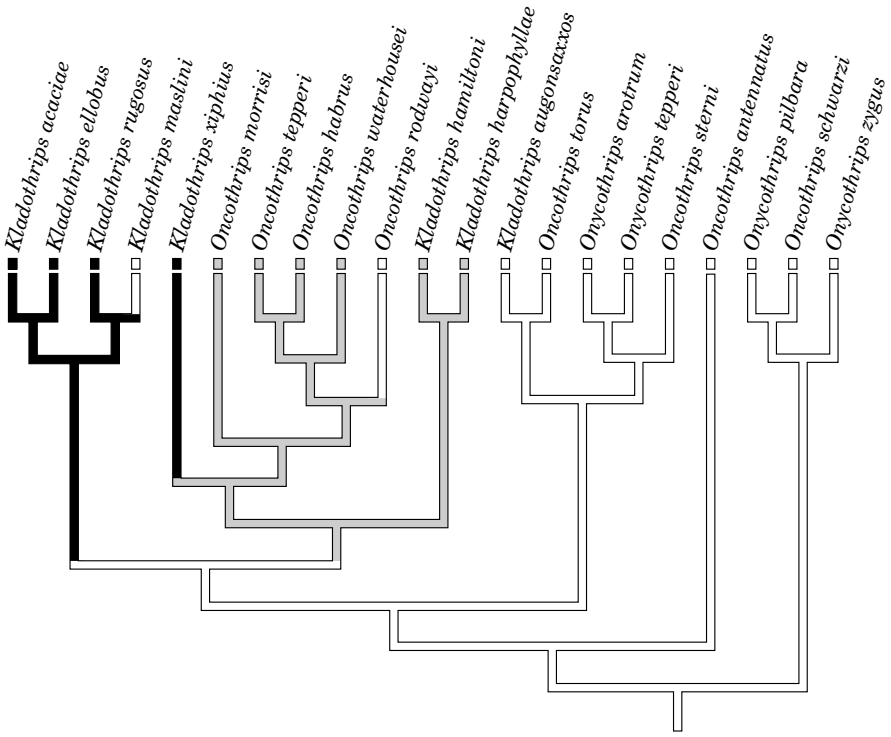

Figure 4. Distribution of life history strategies, mapped on to our most parsimonious tree. White bars indicate species that pupate in the gall, black bars denote species that pupate in the soil and do not have soldiers, and grey bars identify species that have a soldier caste.

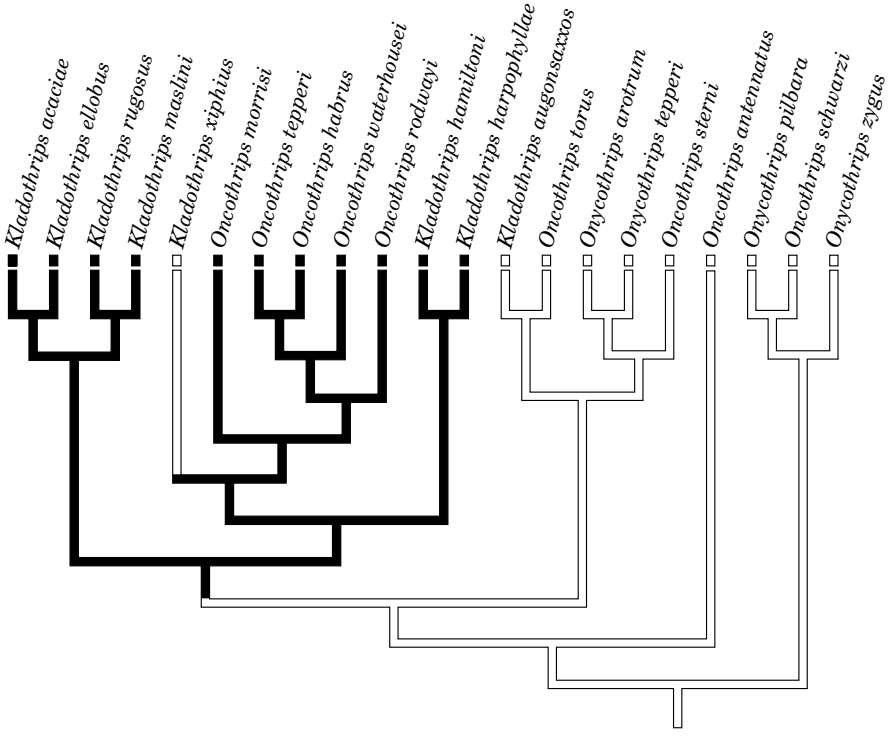

Figure 5. Most parsimonious tree with host plant affiliation mapped for thrips species. White bars indicate a host plant from *Acacia* section Juliflorae, and the black bars denote a host plant from *Acacia* section Plurinerves.

thrips species that invade galls, killing the occupants and using the gall for the purpose of producing their own brood (Crespi & Abbot, 1999). The association of *Koptothrips* with species from the basal lineages on Juliflorae is comparatively low, having only being reported from two species, *Ony. arotrum* and *O. antennatus*. Furthermore, in these species the incidences of parasitism by *Koptothrips* are very rare (Crespi & Abbot, 1999). Among the species with derived life history strategies however, *Koptothrips* have been found to be associated with nearly all taxa except those where insufficient sampling might explain this anomaly (e.g. *Kladothrips maslini*). Thus, it might be that the two derived life history strategies are a direct response to parasitism by *Koptothrips*.

One of the two derived life history strategies involves the development of 'soldier castes'. The soldiers develop rapidly from a small initial clutch of eggs, and are adult when the next clutch of eggs is ready to hatch. Thus the soldiers are able to defend the gall from *Koptothrips* that might attempt to usurp the gall before the second cohort of larvae leave the gall (presumably to pupate in the soil). Another factor common to the species with soldiers is a tendency towards smaller galls (Crespi & Worobey, 1998), which may be an adaptation to avoid the attention of herbivores.

The other derived strategy involves the production of large numbers of eggs that develop rapidly into larvae that leave the gall after the minimum amount of time. These larvae are also assumed to pupate in the soil until environmental cues indicate that the host plants should be producing fresh phyllodes for galling. Unlike the soldier species, these species without soldiers tend to induce larger galls, presumably to allow more larvae to develop within. Brood sizes of species with this strategy tend to be approximately three times larger than that of species with soldiers (Kranz, 2000).

The evolution of life history strategies in gall thrips exhibits remarkable similarities to those in gall aphids: in both taxa, species with soldiers always form galls during part of their life cycle, and gall species with soldiers apparently exhibit longer-lived galls than do gall species without soldiers (Aoki & Kurosu, 1988; Crespi & Mound, 1997; Foster & Northcote, 1994; Moran, 1993; Stern & Foster, 1996; Stern, Whitfield & Foster, 1997). Especially, given that in gall thrips these two life history types are often found on the same trees at the same time, they apparently represent alternative viable solutions the problems of predator and parasite pressure. Strong life history correlates of sociality have been found in other taxa (e.g. Arnold & Owens, 1998), but it is as yet unclear whether longer life cycles and lower fecundity are causes or effects of eusociality, or perhaps both.

COMPARISON OF THE CLASSIFICATION AND PHYLOGENY OF GALL THRIPS

As indicated by our phylogenies, none of the genera with gall-inducing species form monophyletic groups. Previous taxonomic work based on morphological data indicated that there were some groups whose species are closely related and others whose taxonomic position is more tenuous (Mound *et al.*, 1996). The molecular data also supports some of these relationships outlined by Mound *et al.* (1996), such as the grouping of five of the *Oncothrips species* (*O. habrus*, *O. tepperi*, *O. waterhousei*, *O. rodwayi*, *O. morrisi*). However, the remaining species are distributed though the phylogeny to such an extent that they indicate that the existing classification does not reflect the true relationships between either the species or the genera of gall thrips. Unfortunately, the phylogeny does not group the gall-inducing species into three clearly discrete clades corresponding to the existing genera, as might be desired to retain the existing genera. It would appear that renaming the species to fit the molecular phylogeny would render the existing keys and identifying characters useless, but the current classification can be misleading about the evolutionary relationships within the group. As such, it would seem that there are two solutions to this problem. The first solution is to leave the generic classification as it is because it facilitates the recognition of one species from another. The alternative would be to reduce the group of twenty-one species to a single genus. This latter solution would not provide more information about evolutionary relationships but would have the distinct advantage of not being misinformative about these relationships. It is anticipated that this issue will be resolved in future publications.

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