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EVOLUTIONARY DIVERSIFICATION OF AUSTRALIAN GALL-INDUCING THRIPS

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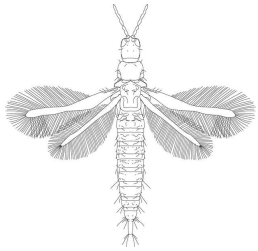

CONTENTS

SUMMARY

This work further elucidates processes involved in promoting and sustaining evolutionary diversification within the gall-inducing thrips that specialise on Australian *Acacia*. A phylogenetic approach was taken to determine modes of diversification available to these insects. The extension and revision of the gall-thrips phylogeny is central to the work and primarily focuses on cryptic populations of the *Kladothrips rugosus* and *Kladothrips waterhousei* species complexes. Parallel diversification, where the radiation of the *K. rugosus* and *K. waterhousei* lineages broadly mirror one another, offered a rare opportunity to test hypotheses of coevolution between gall-thrips and their *Acacia* hosts. In the absence of a reliable host *Acacia* phylogeny, indirect inference of insect/plant cospeciation can be arrived at as these two complexes share the same set of host species. The expectation is that if the phylogenies for the gall-thrips complexes show a significant level of concordance, then cospeciation between insect and host-plant can be inferred. Results indicate that the *K. rugosus* species complex comprise populations at species level. A significant level of phylogenetic concordance between the two species complexes is consistent with gall-thrips lineages tracking the diversification of their *Acacia* hosts. Given the less than strict form of insect/host cospeciation, factors impacting host diversification become important to gall-thrips diversification. Gall-thrips radiated over a period during the expansion of the Australian arid-zone. Cycles of host range expansion and fragmentation during the Quaternary could have played a major role in gall-thrips diversity. An interesting feature of resourse sharing amongst the *K. rugosus* and *K. waterhousei* complex members is the apparent absence of competitive exclusion between them. The persistence of this sympatry over millions of years is an unusual feature and merits further investigation.

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Michael John McLeish
August 2006

“So listen well and hear the call,
and long life to those
who keep the jungle law.”

Rudyard Kipling, 1894.

GENERAL INTRODUCTION

Conspicuous absence of surface water, oscillating drought and flood, extreme temperature, nutrient deficient saline soils, strongly weathered landforms, and millions of years – is the setting of the research presented here. The taxonomic foci are the *Acacia* (Leguminosae), a dominant feature of arid Australia and host to specialist gall-inducing thrips (Insecta: Thysanoptera). Evolutionary conservation of affinities bonding such plant and phytophagous insect groups offer a means of untangling mechanisms driving evolutionary diversification. The theory of coevolution put forward by Ehrlich and Raven (1964) has been one of the more influential explanations for the enormous diversity of phytophagous insects and is a central theme in this work.

Four manuscripts are presented as chapters addressing the following broad questions: (i) is there a reproductive boundary defining two morpho-types that specialise on the same host species; (ii) what systematics describe two species complexes comprising other morpho-types inhabiting different host species and to what extent do insect and insect-plant groups codiverge; (iii) to what degree does host plant diversification facilitate gall-thrips diversification; and (iv) what ecological processes maintain diversity among gall-thrips on *Acacia*?

About 5500 described species of thrips are known throughout the world either living on fungi or green plants exploiting leaves, buds, flowers and some with the ability to form galls. Of these species, approximately 3200 belong to the family Phlaeothripidae in the sub-order Tubulifera. The subfamily Phlaeothripinae comprises approximately 2500 species and at least 250 of these species feed exclusively on *Acacia* foliage (Crespi *et al*., 2004). This group is distributed mainly in Australia, India, and Southeast Asia. Before this work, 22 described thrips species induce galls on phyllodes of Australian *Acacia* (Morris *et al*., 2001). Gall-inducing *Acacia* thrips represent a monophyletic group (Morris *et al*., 2002) implying that the ability to induce galls (see Appendix I) has arisen once. Gall-thrips induce galls on young, actively growing phyllodes (a flat dilated petiole functioning as a leaf) of *Acacia* by the feeding activity of females and fully enclose the founder within a

few days after initiation. The number of individuals that develop in the gall varies between species and can be more than one thousand or as few as twenty. All thrips develop through two mobile larval stages, two or three mobile pupal stages, and eclose as adults (Crespi & Mound, 1997).

Acacia gall-thrips comprise cryptic populations at undefined intermediate stages between polymorphism and full species margins. Polymorphisms, cryptic species, host races, biotypes, and ecological races are various stages in differentiation in a continuum from single to separate species (Bush, 1969). Differentiating between them remains a contiguous area. Cryptic species cannot be distinguished on the basis of morphology and might indicate recently diverged taxa (Walker, 1964; Jaenike, 1981). The *Kladothrips rugosus* (Froggatt) species complex is not grossly distinguishable at the morphological level (Crespi & Worobey, 1998; Mound *et al*., 1996). However, variation in gall morphology within the *K. rugosus* complex rivals interspecific gall-form variation. Gall morphology is not strictly unique at the species level. Evidence of divergence, derived from gall measurements (Crespi & Worobey, 1998) among some of these cryptic populations, raises the question of the degree of differentiation among populations in the *K. rugosus* species complex. To identify important factors propagating diversification in this group, species delimitation, host affiliation, historical biogeography, and ecological considerations are explored using phylogenetic approaches.

Taxonomic formalisation of sympatric gall-thrips morpho-types that belong to the *K. rugosus* species complex and specialise on the same host species, establishes a systematic perspective for these taxa. Phylogenetic inference is used as a platform to test hypotheses of cospeciation and convergence between gall-thrips and *Acacia* hosts. Climatic processes impacting the expansion of Australian arid-zone biomes provide background to explaining gall-thrips diversification in terms of host diversification. Resource sharing among gall-thrips taxa specialising on the same host is then explored using a phylogenetic approach to explain the maintenance of gall-thrips diversity across evolutionary and ecological timeframes. Phylogenetic inferences made in each chapter are generated from varying numbers of taxa depending on the objective pursued in each case.

This thesis presents work in the form of multi-authored manuscripts. The Candidate wholly contributed to the laboratory, analysis, and discussion components presented. The following contributions were made by various co-authors: Chapter I drew on the taxonomic expertise of Dr. Laurence Mound, providing explanations of morphological variation in the focal thrips taxa; elements of the literature review in the Introduction and general discussion in Chapter II were written with some helpful commentary from Professor Bernard Crespi. The first two chapters, as with Chapters III and IV, were written with commentary from my supervisor Associate Professor Michael Schwarz and co-supervisor Dr. Tom Chapman. Funding from grants awarded to Schwarz and Chapman was also recognised by their inclusion as authors.

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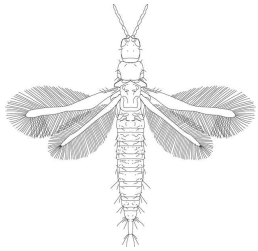

CHAPTER I.

GALL MORPHO-TYPE CORRESPONDS TO SEPARATE SPECIES OF GALL-INDUCING THRIPS (THYSANOPTERA: PHLAEOTHRIPIDAE)

A version of this chapter has been published as:

McLeish, M. J., Chapman, T. W. & Mound, L. A. (2006). Gall morpho-type corresponds to separate species of gall-inducing thrips (Thysanoptera: Phlaeothripidae). *Biological Journal of the Linnean Society*. 88: 555-563.

ABSTRACT

Kladothrips rugosus Froggatt has previously been considered a single polyphagous species that, in Australia, induces galls on several species of *Acacia*, with the gall structure varying both within and between hosts. On *Ac. papyrocarpa*, two types of gall are induced by this species, one with the surface ridged but the other with the surface smooth. Using sequence data from COI and EF-1α gene fragments, we show that the thrips inducing these two gall-types are genetically distinct and comprise separate lineages. Uncorrected "p" distances calculated from COI gene fragments were 0.000 and 0.006 within lineages and 0.074 and 0.078 between lineages. The between-lineage distances are comparable with distances between morphologically distinct species of other *Acacia* gall-thrips. Re-examination of adult thrips from the two gall types revealed consistent differences in body colour as well as in body sculpture. Together with observations on gall founding behaviour, these data indicate that the thrips populations in the two gall types on *A. papyrocarpa* are reproductively isolated and should be considered as separate species. The form from smooth galls on *A. papyrocarpa* is therefore described as *Kladothrips nicolsoni* sp. n., although the form from ridged galls can be considered only as "*K. rugosus* agg." These inconsistencies in the taxonomic status of the various units within the *Kladothrips rugosus* species complex are discussed, although most of them cannot at present be distinguished morphologically.

INTRODUCTION

Where populations occur sympatrically, genetic divergence can indicate reproductive isolation (Dieckmann & Doebeli, 1999). Among Australian thrips there is one lineage of gall-inducing species that are specialist phytophages parasitising only *Acacia* trees, often with overlapping distributions. Gall-thrips have the ability to induce in an *Acacia* phyllode (modified petiole that functions as a leaf) a structure that provides both nutrition and protection against predators and desiccating climatic effects. Gall morphology varies considerably between species (Crespi & Worobey, 1998), and the insect-plant association for the 23 described *Acacia* gall-thrips species is predominantly host-species specific. However, several species are currently considered polyphagous, but possibly comprising host-limited races (Morris *et al*., 2002; Crespi, Morris, & Mound, 2004: 249). Cryptic or sibling species, host races, biotypes, and ecological races can all represent intermediate stages in the processes that lead to full speciation (Bush, 1969; Emelianov, Mallet, & Baltensweiler, 1995; Parsons & Shaw, 2001; Drés & Mallet, 2002). However, these processes have to be viewed within the prevailing ecological conditions. Given the remarkable instability of growing conditions in arid Australia, with rain sometimes not falling for periods of several years, and thus young phyllodes suitable for galling not being available for similar periods (Crespi *et al*., 2004), there are good ecological reasons for considering that some species may be opportunistically polyphagous. A further complication is that at least one of these putatively polyphagous species, *Kladothrips rugosus* (Froggatt), apparently induces two types of gall on the same individual tree, or even on the same phyllode. On *Acacia papyrocarpa* these two gall types have the surface either smooth or strongly ridged (Figure 1). The biological significance of the different gall types is unclear, but the suggestion has been raised that they are induced by sibling species. Alternatively, gall polymorphism might suggest differential resource utilization and niche partitioning imposed by competition (McCoy *et al*., 2001). A direct experimental approach has not proved possible, and so attempts have been made to test the genetic distinctiveness of thrips from different gall types and also different host species.

Species-specificity of gall morphology has been documented in several insect orders (sawflies: Nyman *et al*., 2000: wasps: Cook *et al*., 2002; Stone *et al*., 2002) and broadly accepted as largely under the control of the parasite's genome representing an extended phenotype (Stern, 1995; Crespi & Worobey, 1998; Morris *et al*., 2002; Stone & Schönrogge, 2003). Within *Acacia* gall-thrips species, gall morphology is generally highly conserved and with striking levels of structural diversity exhibited amongst species (Crespi & Worobey, 1998). In contrast, the galls induced on *A. papyrocarpa* are of two discrete types; 1) elongate with smooth surface, henceforth known as "smooth"; and 2) pouched with longitudinal surface ridges, henceforth known as "ridged" (Figure 1). The populations of thrips inducing these two gall types have previously been treated as a single species (Mound, Crespi, & Kranz, 1996; Kranz *et al*., 2000; Crespi *et al*., 2004). However, such an ability by a single species of thrips to induce two different gall types is not consistent with most other *Acacia* gall thrips species that usually induce a gall of invariable structure. This anomaly may be an artifact resulting from our present inability to recognize any morphological differences between the adult thrips involved with these two gall types that might indicate them representing different species.

The name *K. rugosus*, derives from the rugose or wrinkled appearance of the gall from which this species was originally described. Froggatt (1907) illustrated this rugose gall, but did not identify the *Acacia* species on which it had been found. Subsequently, Mound (1971) redescribed *K. rugosus* from material taken at various sites across New South Wales on *Acacia pendula*, but indicated that the galls involved were smooth surfaced, flask-like structures, and that rugose galls were found only occasionally and were "apparently aberrant." In the Australian National Insect Collection, CSIRO Entomology, there are old galls labelled by W.W. Froggatt as *K. rugosus*, and all are rugose pouches with a spiky appearance (Figure 1) as in Froggatt's original illustration. Included with these galls are a few small phyllodes that are more similar to those of *Acacia melvillei* than to phyllodes of *Acacia pendula*. Moreover, *A. melvillei* is known to bear galls with a spiky surface that are induced by thrips of the *Kladothrips rugosus* complex. Thus, the original host

plant of the thrips described by Froggatt is likely to have been *A. melvillei* or a closely related species. Previous work (Mound *et al*., 1996; Kranz *et al*., 2000, Crespi *et al*., 2004) treated *K. rugosus* as polyphagous on several host plants, or suggested that gall polymorphisms on different hosts might be sister-species (Crespi *et al*., 1998; Crespi & Worobey, 1998). DNA sequence data from a population of *K. rugosus* on *Acacia maranoensis* was used to infer the most recent gall-thrips phylogeny (Morris *et al*., 2001), but this population induced non-rugose, elongate, tubular galls reminiscent of the 'smooth' type on *A. papyrocarpa* (personal communication, DC Morris).

Previous work using microsatellite DNA methods (McLeish *et al*., 2006) indicated the possibility of reproductive isolation between the *K. rugosus* populations on *A. papyrocarpa*. A single microsatellite locus revealed inbreeding estimates (F_{IS}) for thrips from the 'smooth' and 'ridged' gall types as different (F_{IS} = 0.09 and F_{IS} = 0.59 respectively) with no shared alleles between 'smooth' and 'ridged' populations at this microsatellite locus (Table 1). This fixed difference is consistent with no gene flow between the two populations, even though both gall types were collected from the same trees (occasionally from the same phyllode). The aim of this study was to use a phylogenetic approach to further evaluate the level of DNA sequence divergence between and within the 'smooth' and 'ridged' gall-thrips populations on *A. papyrocarpa*, and then combine this with a renewed morphological study.

METHODS

Collections

Gall-thrips on *A. papyrocarpa* were collected from sites over three separate locations in South Australia (Table 2). 'Smooth' and 'ridged' gall types were collected from adjacent trees at Middleback Station near Whyalla, only 'ridged' from the Eyre Highway site, and only 'smooth' from the Lake Gilles site. Collections used for the inbreeding estimates (Table 1.) were also from Middleback Station. This sampling scheme enabled comparisons of DNA

sequence and morphological divergence between gall-thrips populations of different types sampled at the same location (Middleback Station), between like-types collected at geographically distant locations (i.e. Middleback samples with either Lake Gillies or Eyre Highway samples), and within like-types (again, Middleback samples with either Lake Gillies or Eyre Highway samples – see Table 2). Four samples collected from these sites were included with DNA sequence data from previous work (Morris *et al*., 2001) to generate the phylogenetic inferences. Voucher specimens of thrips from 'smooth' and 'ridged' type galls have been deposited in the Australian National Insect Collection (ANIC) at CSIRO Entomology in Canberra.

DNA extractions, PCR, and sequencing

The DNA extractions used thrips tissue kept at -80°. To maximise DNA yield each tissue extraction comprised all individuals in a single gall, the brood of one female, using a GENTRA SYSTEMS DNA Extraction Kit. Amplifications of DNA to be sequenced was undertaken using the following protocol: 94°C, 45 sec denaturation; 48°C, 1 min annealing; 72°C, 1 min extension for 34 cycles; with a final cycle of 72°C, 6 min extension. Amplitaq Gold (ROCHE) was used and required a 90°C, 9 min incubation period for the first cycle only. The PCR mixture was a 25µl reaction: 1x buffer (ROCHE), 1 unit of *Amplitaq Gold* polymerase. 4mM of $MgCl_2$, 0.8 mM of dNTPs, 5 pmol of each primer, and unknown concentrations of template DNA. Gene regions from *cytochrome oxidase I* (COI) and *elongation factor - one alpha* (EF-1α) were sequenced. Approximately 1200 bases of the COI gene were amplified using two primer pair sets: LCO1490 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' with HCO2198 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Folmer *et al*., 1994) and C1-J-2183 5'-CAA CAT TTA TTT TGA TTT TTT GG-3' (Simon *et al*., 1994) with A2735 5'-AAA AAT GTT GAG GGA AAA ATG TTA-3' (unpublished work, Crespi B). Approximately 500 bases of the EF-1α gene fragment were amplified using M51.9 5'-CAR GAC GTA TAC AAA ATC GG-3' (Cho *et al*., 1995) with G346 5'-AGA CTC AAC ACA CAT AGG TTT GGA C-3' (unpublished work, Morris D). SeqEd version 1.0.3 was used for editing and creation of consensus sequences. The alignment of new

sequences with the most recent gall-thrips sequence dataset (Morris *et al*., 2001) was straightforward. The dataset of Morris and colleagues (2001) comprised additional 16S and *wingless* gene sequence data that was edited for some of the analyses (see below). Sequences of the COI and EF-1α gene regions are stored at Genbank (accession numbers AY827474-AY827481).

Phylogenetic analysis

Maximum parsimony analysis was carried out using PAUP*b4.10 (Swofford, 2002). The dataset comprised COI, EF-1α, *wingless*, and 16S gene fragments. A bootstrap heuristic search using 1000 bootstrap replicates, tree bisection/reconnection (TBR) branch swapping and random addition of taxa (100 replicates per search and 10 trees held at each step) was used to generate the MP phylogeny. Although parsimonious approaches are relatively assumption-free they can be problematic due to the homoplasious effects of accumulated substitutions in lineages resulting in long branch attraction (Page & Holmes, 1998). Bayesian approaches allow the incorporation of specific evolutionary models to account for the variation in heterogeneous base composition and substitution rate characteristics.

Using a Bayesian framework, Lin and Danforth (2004) reviewed the recent gall-thrips COI, EF-1α, and *wingless* sequence datasets (Morris *et al*., 2001) and its suitability to combined gene region analysis. They showed that A-T rich COI 3rd codon positions are subject to saturation effects and that the differential substitution characteristics between and within COI and EF-1α genes can lead to poor phylogenetic interpretations. Only COI and EF-1α genes were sequenced for the 'smooth' and 'ridged' gall type populations. We used Modeltest 3.0 (Posada & Crandall, 1998) to find the most appropriate substitution model for each of the 1st, 2nd, and 3rd codon positions of both COI and EF-1α gene fragments. These tests showed that different models described the substitution dynamics for different codon positions of our COI and EF-1α dataset. Furthermore, the Bayesian analyses did not respond well to substantial omissions of sequence data, and necessitated the removal of 16S and *wingless* from the analysis. Therefore, the data was divided into six separately modelled partitions comprising 1st, 2nd, and 3rd codon positions of the COI fragment and

1st, 2nd, and 3rd codon positions of the EF-1α fragment. As over-parameterisation can be problematic (Schwarz *et al*., 2004), we limited our a priori choices by applying a general time reversible (GTR) DNA substitution model with invariant gamma rates applied across a proportion of sites with the remaining sites drawn from a gamma distribution (GTR + I + G model). MrBayes 3.0b4 (Huelsenbeck & Ronquist, 2001) was used to infer an ultrameric tree, with divergence times estimated indirectly as the amount of sequence change. Posterior probabilities and mean branch lengths were derived from a consensus of 2000 trees taken from generations 2-3 million, sampling every 500th generation. We ran the Bayesian analysis three times to confirm the repeatability of the outcome.

To estimate levels of genetic distance between and within gall-types, pairwise uncorrected "p" distances for the COI gene region were calculated using PAUP*b4.10 (Swofford, 2002). Distance values are not intended here as a means of identifying different species, a problematic approach for species depiction (Ferguson, 2002), but as useful descriptors of genetic variation. The EF-1α gene fragment did not display adequate sequence divergence to be suitable as a species-level delimitation tool. *Dactylothrips* and *Rhopalothripoides* were the outgroups used (Morris *et al*., 2002).

RESULTS

Phylogenetic outcomes

The MP and Bayesian trees (Figures 2 & 3 respectively) both grouped the ‘smooth’ and ‘ridged’ gall types with the *K. rugosus* population on *A. maranoensis* as sister-taxa. Although the parsimonious outcome for this association lacked resolution, all three Bayesian trees showed the same very high level of support for most bifurcations including the clade containing ‘smooth’’ and ‘ridged’ gall types. Lack of parsimony informative gene characters (11% of the 546 EF-1α sites compared to 26% of 949 COI sites) might explain the low level resolution between ‘smooth’ and ‘ridged’ gall types and some deeper divergences in the MP tree. This result is consistent

with not having sampled 16S and *wingless* DNA from the 'smooth' and 'ridged' gall types. However, the overall resolution produced by our Bayesian analysis mirrors the most recently published gall-thrips phylogenies (Morris *et al*., 2001) that used COI and EF-1α fragments as well as 16S and *wingless* gene regions.

Gene flow between like gall-types was expected to be greater than between the different morphs. Parsimony and Bayesian trees show the 'smooth' gall-type populations at different sites as sister-taxa. This was also true for the 'ridged' gall-type populations at different sites. Additionally, the ultrameric tree (Figure 3) indicated negligible amounts of sequence divergence between 'smooth' gall-type populations and between 'ridged' gall-type populations. Pairwise uncorrected "p" distances for 1236 bases of the COI gene (Table 3) between like gall-type populations at different sites ('ridged' - 0.6% and 'smooth' - 0.0%) are indicative of relatively high levels of genetic exchange. Distances between the 'smooth' gall type and 'ridged' gall type infesting *A. papyrocarpa* (ranged from 7.4% to 7.8%) indicate levels of divergence comparable to that among other gall-thrips species (Morris *et al*., 2001). Distances between each of the 'smooth' and 'ridged' gall-types and the outgroup taxon, *Rhopalothripoides*, were in the order of 12%, which is consistent with other outgroup/ingroup estimates for gall-thrips (Morris *et al*., 2001).

Morphological differences

Specimens from the two types of gall were mounted onto microscope slides in a standard manner. Females from 'ridged' galls on *A. papyrocarpa* were found to be sharply bicoloured with the metathorax and abdominal segments I – III yellow. In contrast, females from 'smooth' galls on *A. papyrocarpa* were found to be a unicolourous brown. Despite this colour difference, details of body structure and chaetotaxy were remarkably constant between the thrips from the two gall types, and the only consistent difference between them that was found was in the form of the sculpture on the second and third abdominal tergites. Adults from smooth galls have the sculpture formed of uniformly sized reticulations posterior to the median campaniform sensilla. In contrast, adults

from ridged galls have some of the sculptured reticles in this position wider than long (Figure 4).

These differences in body colour and sculpture were present in specimens collected over three consecutive years from the same location. Therefore, genetic differentiation does not appear to be based on natural selection acting on a single generation (Jaenike, 1981) and interbreeding between the two populations does not appear likely. Ridged galls were each found to contain a single foundress, whereas smooth galls commonly showed a high rate of co-founding, with two adults present in the young galls. A female and a male were identified together in each of 15% (n = 21) of 'smooth' galls and 0% (n = 27) of 'ridged' galls. The differences in colour, sculpture and biology, together with the molecular evidence presented above, all indicate that the populations from ridged and smooth galls on *A. papyrocarpa* should be treated as distinct species. The species from the smooth galls is therefore described here as a new species.

Kladothrips nicolsoni sp. n.

A full technical description of this species is given, under the name *K. rugosus*, in Crespi *et al*. (2004: 249). The only differences from that species are that abdominal segments I – III are as brown as IV – VII (Figure 1), the metathorax is scarcely paler than the brown mesothorax and prothorax, and the sculptured reticles on the posterior half of tergites II – III are all small and equiangular (Figure 4). These character states are consistently present in both sexes.

Holotype female: South Australia, Middleback (near Whyalla), from smooth gall on phyllodes of *Acacia papyrocarpa*, 25.ii.2002 (M. McLeish), in Australian National Insect Collection, CSIRO Entomology, Canberra.

Paratypes: 8 females 5 males with same data as holotype.

DISCUSSION

The evidence suggests that two gall types on *A. papyrocarpa*, 'ridged' and 'smooth', are induced by different species. Although it has proved possible to

provide a name for the species from smooth galls, the species from ridged galls remains equivocal and is referred to below as "*K. rugosus* agg.". The question of species level identities within this aggregate requires further study. Current molecular work suggests that some members of the aggregate may be genetically distinct, although no morphological characters have yet been found to distinguish them. This lack of morphological divergence has evident problems for traditional taxonomy. However, we suggest that there is no inherent reason why different thrips species that live enclosed within galls must be morphologically distinct, given that there is likely to be little selection pressure on their body form arising from differences between the within-gall habitats. Indeed, we suggest that "morpho-taxonomy" is little more than an historical artifact in the methodology of species recognition, despite commonly providing the most practical methods.

The phylogenetic inferences presented here (Figures 2 & 3) are consistent with the most recently published gall-thrips phylogenies (Morris *et al*., 2001). *Kladothrips nicolsoni* and the species from the 'ridged' gall type on *A. papyrocarpa* form a monophyletic clade with the "*K. rugosus* agg." population on *A. maranoensis*, although this clade also includes *K. maslini*, a species in which the adults are similar in structure but in which the galls are dagger-like cones on *Acacia orites* (Crespi *et al*., 2004). Within-population sequence divergences for each of the 'smooth' and 'ridged' species was negligible indicating that substantially more genetic exchange had occurred between like gall-types. Both species on *A. papyrocarpa*, and *K. rugosus* on *A. maranoensis*, fall into separate lineages. Pairwise genetic distances between these lineages (Table 3) indicate a level of divergence comparable to that among other gall-thrips species. Additionally, differences in inbreeding estimates between the 'smooth' and 'ridged' species, and the presence of a fixed difference in the microsatellite locus analysed (Table 1), supports strongly the premise that these species have had negligible gene flow between them for a long period. Lower estimates of inbreeding from thrips in smooth galls (F_{IS} = 0.09) are compatible with relatively high rates of cofounding (15% compared to 0% of the 'ridged' galls) as outbreeding is more likely. The tergite sculpture and colour differences, incompatible founding biology, and

asynchronous periodicity of gall types between seasons all indicate that these are different species. The different periodicity between the two from successive seasons suggests that they are independent and supports the conclusion that they are separate species.

Diversification in plant-insect systems is usually thought to be a product of either joint cladogenesis of host and parasite via cospeciation (Page, 1994), or a host shift. The appearance of multiple gall-thrips species on *A. papyrocarpa* might be explained by a host-plant shift, where one or more populations have united on the same host species after some period of reproductive isolation (Ehrlich & Raven, 1964; Cronin & Abrahamson, 2001; Nyman, 2002; Stone & Schönrogge, 2003; Crespi *et al*., 2004). Highly specialised modes of feeding of galling insects are believed to be responsible for the conservative restrictions to single host plant species (Mound, 1994; Stern, 1995; Ward *et al*., 1998). This is true for the preference gall-thrips show for their *Acacia* hosts. The distribution of *A. papyrocarpa*, from near Kalgoorlie in Western Australia (WA) across the Nullarbor to the southern Flinders Ranges in South Australia, is relatively broad compared with other *Acacia* species that support gall-thrips. The distribution of another closely related arid zone gall-thrips host species, *A. aneura*, overlaps that of *A. papyrocarpa*. Therefore, it is expected that a predominance of traits should be phylogenetically conserved between these two species (Gaston, 1998; Crisp *et al*., 2001). *Acacia aneura* displays high levels of genetic variation over its enormous range-size and is thought to be partly the result of hybridisation, apomixis and polyploidy (Miller, Andrew, & Maslin, 2002; Andrew *et al*., 2003). A host shift becomes feasible under certain conditions given genetic variation in both host and insect makes utilisation of closely related species possible. Host specialisation may select for the evolution of ecological specialisation in habitat use and therefore a potential for reproductive isolation even between sympatric groups (Ward *et al*., 1998; Kelley *et al*., 2000; Cronin & Abrahamson, 2001).

Other known examples (Crespi *et al*., 2004) of sympatric eco-types supported by the same host (tree) species include morphs on *Acacia tephrina* and *Acacia microcephala* where each host species has both elongate-smooth

and pouched-spiky gall types. Up to 10 other host races parasitising different *Acacia* host species are currently recognized as the *Kladothrips rugosus* species complex (Mound *et al*., 1996; Kranz *et al*., 2000, Crespi *et al*., 2004) or as equidistant sister-species (Crespi *et al*., 1998; Crespi & Worobey, 1998). The *K. rugosus* host races might comprise populations that have recently undergone a major radiation at various levels of reproductive isolation, and therefore, important candidates for exploring mechanisms generating diversity in gall-inducing thrips. Geographically fragmented and restricted relictual taxa believed to have been caused by cyclic expansion and contraction of xeric zones during the late Tertiary is thought to have facilitated the radiation of *Acacias* during the Pleistocene (Byrne *et al*., 2002; Andrew *et al*., 2003). A recent radiation of *Acacia* and gall-thrips offers the opportunity to tease apart host-switching and host-tracking events not complicated by multiple shifts over relatively long periods that can obscure the recognition of such processes. The taxonomic boundaries among these *K. rugosus* host races are unknown. These distinctions are important as behavioural work and understanding gall thrips radiation demands accurate resolution between taxa to make accurate assessment of the biological processes operating. The fidelity of gall structure as an extended phenotype (Crespi & Worobey, 1998) for each race or species at lower taxonomic levels combined with well-supported phylogenetic inference will be useful for understanding the mechanisms generating diversity in Australian gall thrips on *Acacia*.

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Table 1: Microsatellite DNA locus analysis showing the microsatellite primer, inbreeding estimate (F_{IS}), the number of galls and individuals sampled, and the frequency of the most common allele in each morph shown in square brackets. MB designates the collection site at Middleback Station.

Taxon	Primers	F_{IS} (SE)	no. of galls/ individuals	Allele lengths [freq]
'smooth' MB	rugAAT1	0.09 (0.25)	21/21	121, 124 [0.71].
'ridged' MB	rugAAT1	0.59 (0.16)	27/27	134, 137 [0.63], 140, 143.

Table 2: Description and locations of collection sites of gall thrips on *Acacia papyrocarpa*. MB = Middleback Station, EH = Eyre Highway, and LG = Lake Gilles (MB > 35 km to LG and MB > 450 km to EH).

Taxon gall type	Host	Site description	Date
smooth & ridged MB	*A. papyrocarpa*	20 km W of Whyalla SA	Feb 02
ridged EH	*A. papyrocarpa*	35 km W of Yalata SA	Feb 03
smooth LG	*A. papyrocarpa*	35 km SW of Iron Knob SA	Feb 03

Table 3: Pairwise uncorrected "p" distances for 1245bp of COI mtDNA among morphs of the 'smooth' and 'ridged' gall types found on *Acacia papyrocarpa* (pap) and *K. rugosus* from the host *Acacia maranoensis*. Outgroup distances given by comparison with *Kladothrips* sister-genus *Rhopalothripoides*. Within gall-type/host population distances are highlighted in bold. MB = Middleback Station, EH = Eyre Highway, and LG = Lake Gilles.

Taxon	*K. rugosus* (mar)	**'ridged' EH (pap)**	'ridged' MB (pap)	**'smooth' LG (pap)**	'smooth' MB (pap)
'ridged' EH (pap)	0.063				
'ridged' MB (pap)	0.057	**0.006**			
'smooth' LG (pap)	0.083	0.074	0.078		
'smooth MB (pap)	0.086	0.075	0.078	**0.000**	
Rhopalothripoides	0.127	0.117	0.129	0.122	0.129

Figure 1: Thrips and galls of the *K. rugosus* group. Adult and gall from *Acacia melvillei* (A & B), adult and 'ridged' gall from *Acacia papyrocarpa* (C & D), and *Kladothrips nicolsoni* and 'smooth' gall from *A. papyrocarpa* (E & F).

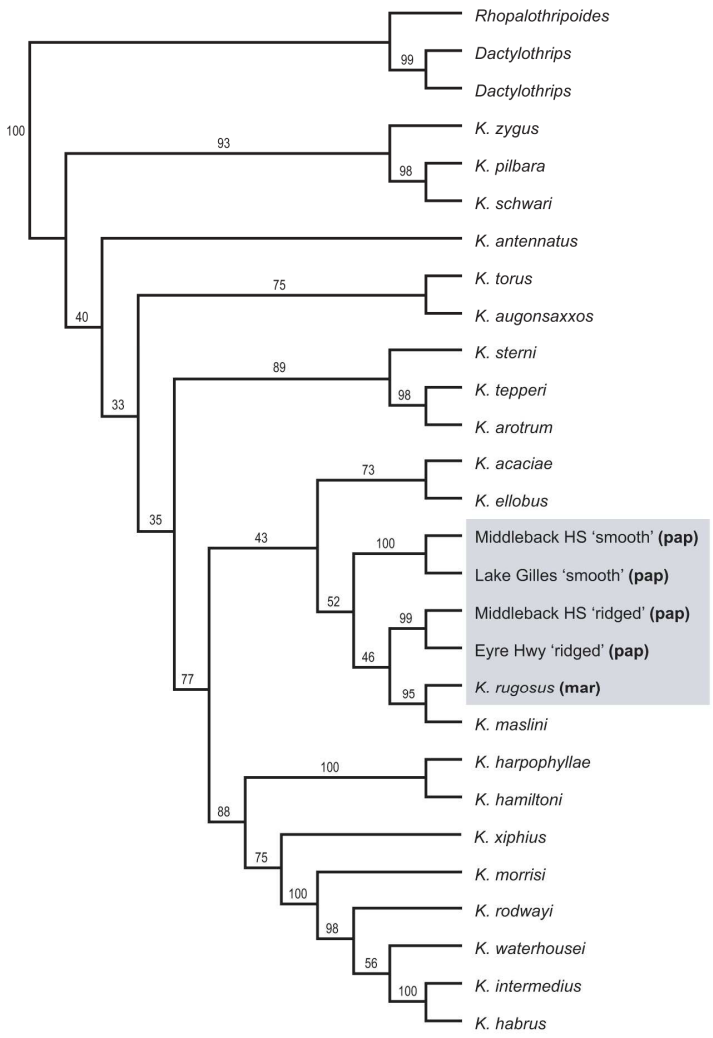


Figure 2: A consensus tree using maximum parsimony, COI, EF-1α, *wingless*, and 16S genes, a heuristic search with tree bisection-reconstruction (TBR) branchswapping, random addition of taxa, 100 replicates per search, 10 trees held at each step, and 1000 bootstrap replicates. 'Smooth' and 'ridged' gall types are indicated with a grey box.

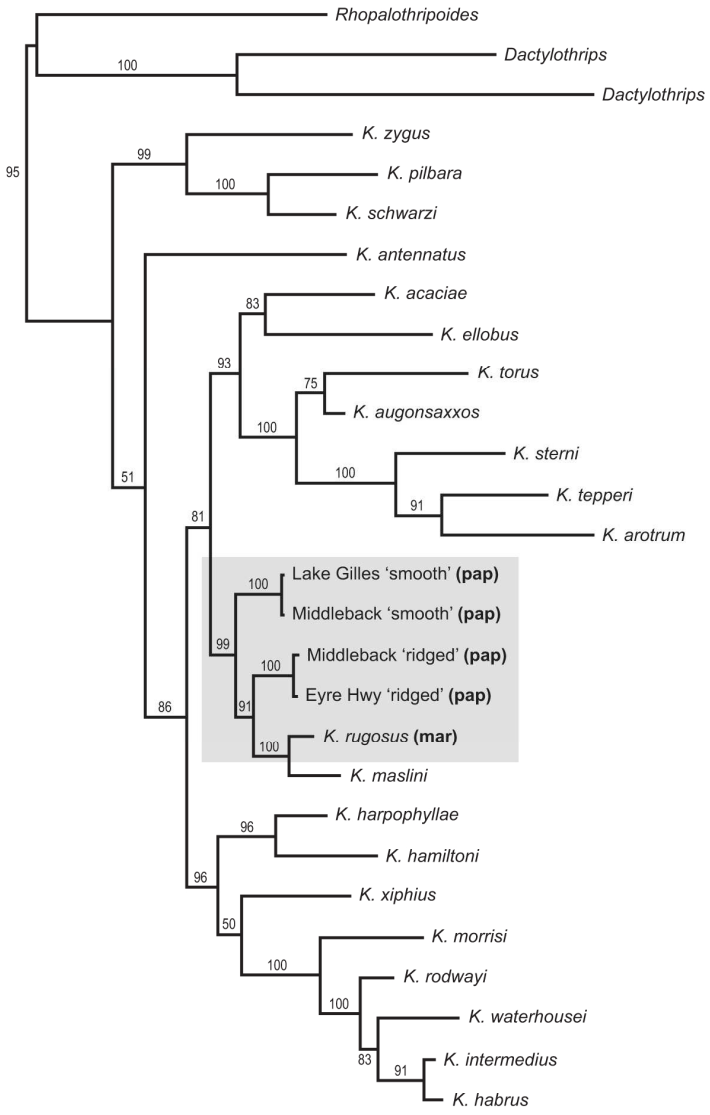


Figure 3: A Consensus ultrameric phylogram from a Bayesian analysis where gene regions were separated into six partitions comprising 1st, 2nd, and 3rd codon mitochondrial positions and 1st, 2dn, and 3rd codon EF-1α positions. Posterior probabilities and mean branch lengths are derived from 2000 trees taken from generations 2-3 million, sampling every 500th generation. Five *Kladothrips rugosus* morphs are indicated with a shaded rectangle. The 'smooth' and 'ridged' morphs are given by collection site and gall type. Host trees are indicated in brackets *pap* = *Acacia papyrocarpa* and *mar* = *Acacia maranoensis*.

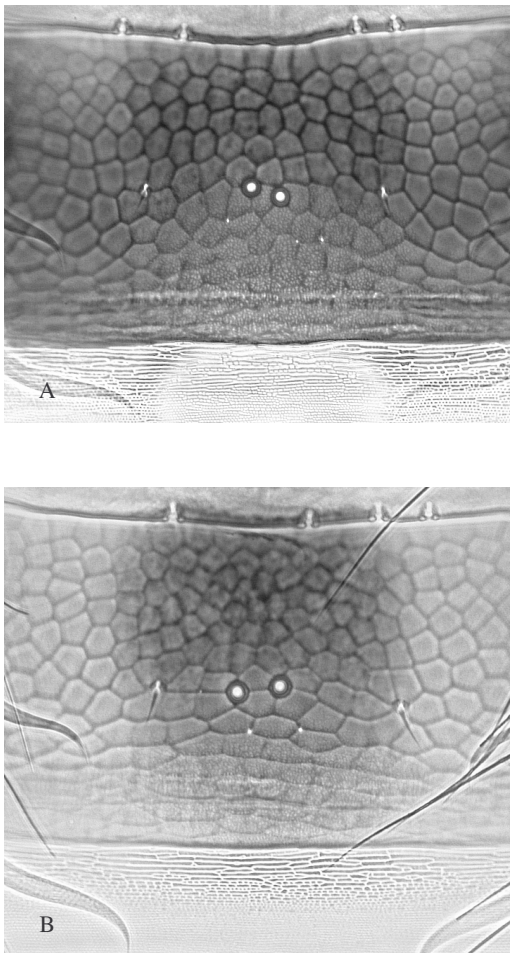
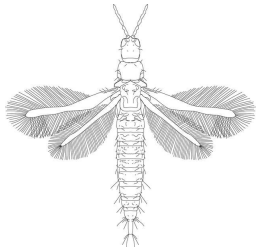


Figure 4: Third abdominal tergite, *Kladothrips nicolsoni* (A) and third abdominal tergite, *K. rugosus* (B). Adults from smooth (*K. nicolsoni* sp. n.) galls have the sculpture formed of uniformly sized reticulations posterior to the median campaniform sensilla while those from ridged galls have some of the sculptured reticles in this position wider than long.



CHAPTER II.

PARALLEL DIVERSIFICATION OF AUSTRALIAN GALL-THRIPS ON *ACACIA*

A version of this chapter has been published as:

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ABSTRACT

The diversification of gall-inducing Australian *Kladothrips* (Insecta: Thysanoptera) on *Acacia* (Leguminosae) has produced a pair of sister-clades, each of which includes a suite of lineages that utilize virtually the same set of 15 closely-related host plant species. This pattern of parallel insect-host plant radiation may be driven by cospeciation, host-shifting to the same set of host plants, or some combination of these processes. We used molecular-phylogenetic data on the two gall thrips clades to analyze the degree of concordance between their phylogenies, which is indicative of parallel divergence. Analyses of phylogenetic concordance indicate statistically-significant similarity between the two clades. Their topologies also fit with a hypothesis of some degree of host-plant tracking. Based on phylogenetic and taxonomic information regarding the phylogeny of the *Acacia* host plants in each clade, one or more species has apparently shifted to more-divergent *Acacia* host-plant species, and in each case these shifts have resulted in notable divergence in aspects of the phenotype including morphology, life history and behaviour. Our analyses indicate that gall thrips on Australian *Acacia* have undergone parallel diversification as a result of some combination of cospeciation, highly restricted host-plant shifting, or both processes, but that the evolution of novel phenotypic diversity in this group is a function of relatively few shifts to divergent host plants. This combination of ecologically restricted and divergent radiation may represent a microcosm for the macroevolution of host plant relationships and phenotypic diversity among other phytophagous insects.

INTRODUCTION

Evolutionary conservation of associations between plant and phytophagous insect groups is a central theme in biology and provides a platform for testing hypotheses rich in scope (Futuyma & Moreno, 1988; Jermy, 1993; Kelley *et al*., 2000; Craig *et al*., 2001; Johnson *et al*., 2003; Nyman, 2002; Ward *et al*., 2003). Coevolution theory (Ehrlich & Raven, 1964) was the historic impetus driving work endeavouring to penetrate factors explaining radiations of both phytophagous insects and their host-plants via selective responses to one another over a relatively long period. 'Coevolution' has also been used to demonstrate joint speciation of interacting lineages, or cospeciation (Herre *et al*., 1996; Clayton *et al*., 1999; Page, 2003). However, the extent to which phytophagous insects and the plants with which they interact exert selection on one another is complex, highly varied among lineages, and unclear (Jermy, 1984, 1993; Ballabeni *et al*., 2003). In this study we infer a phylogeny of gall-inducing thrips on Australian *Acacia* and test hypotheses concerning how this plant-insect assemblage has evolved.

Gall-inducing insects are tightly constrained to mechanisms by which speciation might proceed. Australian gall-inducing thrips are phytophagous insects that have evolved strategies permitting specific utilisation of desert *Acacia* species for food and shelter and for these reasons gall induction imposes a level of phylogenetic constraint (Cornell, 1983; Jermy, 1993; Farrell & Mitter, 1998a; Craig *et al*., 2001; Ward *et al*., 2003). Host race formation is apparent in gall-thrips on *Acacia* (Crespi *et al*., 2004). As well as preadaptation to closely related host plants, cospeciation and host switching across related plants has been shown to result in life history shifts, host specialisation, and the macroevolutionary conservatism in resource use (Ehrlich & Raven, 1964; Berlocher, 2002; Crespi *et al*., 1998; Després & Jaeger, 1999; Cronin & Abrahamson, 2001; Drès & Mallett, 2002; Crespi *et al*., 2004).

Gall-inducing thrips are a monophyletic group that inhabit species of *Plurinerves*, *Juliflorae*, and *Phyllodinae Acacia* subgenera, or sections. Putative gall-thrips species on closely related hosts are of particular interest. Presumably, these taxa have recently diverged and are expected to include taxa

near or below species level and provide a more transparent interpretation of cladogenesis in gall-thrips with fewer extinction events obscuring thrips-*Acacia* associations. *Kladothrips rugosus* (Froggatt) and *Kladothrips waterhousei* (Mound & Crespi) induce galls on the same 14 *Plurinerves* host species showing a high degree of distribution overlap. The phylogenetic relationships among these cryptic taxa are yet to be resolved. Cryptic species are different species that cannot be easily distinguished on the basis of morphology and is indicative of recently diverged species (Jaenike, 1981; Parsons & Shaw, 2001). The apparent cryptic species *K. rugosus* and *K. waterhousei* complexes appear overwhelmingly host-specific, they induce disparate taxon-specific gall morphologies, and preliminary molecular work using COI sequence data and microsatellites have provided strong evidence for species-level divergence among them (McLeish *et al*., 2006). However, some of these putative species show little genetic divergence, which is suggestive of host race population's status.

The scope of this work does not include discussion of species definitions, but contends that levels of polymorphism, below that of species, exist in our dataset and require elaboration. Genetic distances among *K. rugosus* and among *K. waterhousei* populations show that a large majority of the *K. rugosus* and *K. waterhousei* complex members are apparently different species, though additional diagnoses would be useful. Measures of gene flow have to be determined to show reproductive isolation. In addition to genetic distance, it is also crucial to use behavioural and ecological criteria to identify species (Ferguson, 2002). Both the phylogenetic inferences indicate gall structure is highly conserved amongst all newly sampled populations. It is commonly accepted that gall morphology is largely under the control of the insect genome and represents an extended phenotype (Stern, 1995; Crespi & Worobey, 1998; Morris *et al*., 2002; Stone & Schönrogge, 2003). Fidelity of gall structure over different host species is consistent with gall phenotype being largely determined by the thrips genotype and therefore a potentially useful diagnostic character in species identification. Species-specificity of gall morphology is evident in other insect orders (sawflies: Nyman *et al*., 2000: wasps: Cook *et al*., 2002). Recent molecular work has shown two *K. rugosus*

populations, each of which induces a discrete gall-type, once believed to be the same species, are different (McLeish *et al*., 2006).

The *K. rugosus* and *K. waterhousei* complexes thus appear to represent ecological replicates (for a definition see: Johnson & Clayton, 2002) sharing the same set of host species, each expected to cluster into a separate clade and respond in parallel to host speciation via cospeciation, host switching and/or host race formation. These clades thus represent an excellent opportunity to test for parallel diversification and evaluate the roles of historical contingency and selection in evolutionary change.

Modes of speciation

Speciation in gall-thrips might proceed by the formation of host-related races where there is reduced gene flow among populations of a single species parasitising two or more localised host species leading to reproductive isolation (Jaenike, 1981, 1990; Emelianov *et al*., 1995; Parsons & Shaw, 2001; Drés & Mallet, 2002). Speciation via a host shift can be thought of as a transition from polymorphism (e.g. for host preference) to host race preceding a transition from host race to reproductively isolated species. Host races are maintained by reduced gene flow predominantly via differential host preference. By contrast, host-related sibling species are reproductively isolated for reasons in addition to differential host preference (Jaenike, 1981). Genetic divergence data suggests that host-related races of gall-thrips are actually a series of host specific sibling-species, which is consistent with the strong host-plant specificity shown in virtually all other gall-inducing insects (Crespi *et al*., 2004; Rohfritsch & Shorthouse, 1982).

Cospeciation between phytophagous insects and their hosts, parasites, or mutualists has been clearly demonstrated in a number of cases, most of which involve strong host-plant specificity and intimate insect-plant relationships such as gall-induction or complex physiological and life-history adaptation (Ronquist & Nylin, 1990; Baker, 1996, Herre *et al*., 1996; Machado *et al*., 1996; Roderick, 1997; Roderick & Metz, 1997; Farrell & Mitter, 1998b; Burckhardt & Bassett, 2000; Clark *et al*., 2000; Itino *et al*., 2001; Weilben & Bush, 2002; Weilben, 2004). The majority of studies, however, indicate that

congruence between insect and host plant phylogenies is partial or nonexistent, and thus host-shifting appears to be the more prevalent mechanism in determining the associations of insects and their hosts (Humphries *et al*., 1986; Weintraub *et al*., 1995; Janz & Nylin, 1998; Dobler & Farrell, 1999; Janz *et al*., 2001; Jones, 2001; Lopez-Vaamonde *et al*., 2001; Ronquist & Liljeblad, 2001). Consequent to host shifting, fitness tradeoffs between hosts, or ecological divergence of derived, host-shifted populations, may spur the evolution of reproductive isolation (Joshi & Thompson, 1997; Hawthorne & Via, 2001; Nosil *et al*., 2002), and colonisation of new host-plant lineages may provide opportunities to diversify rapidly (Ehrlich & Raven, 1964; Mitter *et al*., 1988; Farrell & Mitter, 1998b).

In this paper we test for parallel speciation in the *K. rugosus* and *K. waterhousei* species complexes, and evaluate hypotheses for their joint diversification on Australian *Acacia*. To do so, we first extend and revise the current gall-thrips phylogeny (Morris *et al*., 2001) with addition of *K. rugosus*, *K. waterhousei*, *Kladothrips habrus*, and *K. intermedius* 'races' from different Acacia species; and second, use the phylogeny to test for parallel patterns of diversification between the *K. rugosus* and the *K. waterhousei* groups, which would be indicative of cospeciation of each of these groups with their *Acacia* hosts, or the parallel evolution of the same set of host-plant shifts.

Cospeciation between gall-inducing thrips and host *Acacia* lineages has been suggested at a macroevolutionary scale in an explicitly phylogenetic context (Crespi *et al*., 2004). Two thrips lineages, each producing a morphologically discrete, elongate or pouched gall type on *Acacia* sections *Plurinerves* and *Juliflorae* can be traced from two ancestral gall-inducing species on a single ancestral *Acacia* lineage. Both derived thrips lineages have retained the ancestral elongate-pouched gall type combination. A hypothesis of cospeciation makes three predictions (Crespi *et al*., 2004): 1) phylogenies of parallel thrips lineages should be identical or very similar to each other; 2) phylogenies of thrips lineages should be identical or very similar to that of the host plants; and 3) speciation events among parallel thrips lineages and host lineages should be contemporaneous.

METHODS

Collections, DNA extractions, PCR, and sequencing

Taxa belonging to the *K. rugosus*, *K. waterhousei*, *K. intermedius*, *K. habrus*, and *K. sterni* species complexes (Crespi *et al*., 2004) were collected from widely distributed *Acacia* populations across Australia (Table 1). Complementary DNA sequence data derived from these taxa were added to the dataset of Morris and colleagues (2001). Voucher specimens of these taxa have been deposited in the Australian National Insect Collection (ANIC) at CSIRO Entomology in Canberra. Gall morphology is highly conserved within each gall-thrips taxon with structural diversity exhibited amongst them (Crespi & Worobey, 1998), and was used in conjunction with host species identification (Maslin, 2001) to discriminate amongst gall-thrips races. To test whether gall structure can be used as an indicator of association between like-types, we mapped three distinct gall structure categories onto focal taxa in our phylogenies: (1) 'spiky' galls have very obvious pointed protrusions: (2) 'elongate' galls are those that are elongate or tubular, some of which have subtle surface textural qualities such as fine striations: and (3) 'pouched' galls that form from a 'ballooning' of petiole tissue from one surface of the phyllode with a noticeably more narrow ostiole than is formed in elongate galls. 'Spiky' and 'pouched' gall-types tend to spheroid dimensions and can include in their surface architecture the presence or absence of longitudinal ridges or spiky projections. These three classifications are not intended as strict diagnostic traits, rather a convenient means of broadly discerning different races that specialise on the same host species.

Fragments of *cytochrome oxidase I* (COI), *elongation factor - one alpha* (EF-1α), *wingless*, and 16S (ribosomal RNA) gene regions were sequenced. The DNA extractions used for the sequencing data were from fresh tissue frozen to -80°. To maximise DNA yield each tissue extraction comprised of all individuals in a single gall, the brood of one female (Chapman *et al*., 2000), using a GENTRA SYSTEMS DNA Extraction Kit. Amplifications of DNA was undertaken using the following protocol: 94°C, 45 sec denaturation; 48°C, 1 min annealing; 72°C, 1 min extension for 34 cycles; with a final cycle

of 72°C, 6 min extension. The polymerase enzyme used was Amplitaq Gold (ROCHE) that required a 90°C, 9 min incubation period for the first cycle only. The PCR mixture was a 25µl reaction including: 1x buffer (ROCHE), 1 unit of *Amplitaq Gold* polymerase. 4mM of $MgCl_2$, 0.8 mM of dNTPs, 5 pmol of each primer, and unknown concentrations of template DNA.

The following primer pairs were used to amplify the various gene fragments. The COI gene fragment was amplified using two primer pair sets: *LCO1490*: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' with *HCO2198* 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Folmer *et al*., 1994) and *C1-J-2183* 5'-CAA CAT TTA TTT TGA TTT TTT GG-3' (Simon *et al*. 1994) with *A2735* 5'-AAA AAT GTT GAG GGA AAA ATG TTA-3' (Crespi B). The EF-1α gene fragment was amplified using primer pair sets *M51.9* 5'-CAR GAC GTA TAC AAA ATC GG-3' (Cho *et al*., 1995) with 5'-AGA CTC AAC ACA CAT AGG TTT GGA C-3' (Morris D) and G730 5'-ACC TTC GCT CCT GCC AAC TT-3' with G731 5'-AAG GGT GAT AAT AGC AGC-3' (McLeish M). The *wingless* gene fragment was amplified using primer pair 5'-TAG ACG TAT CGT TAC ACT GC-3' and 5'-CGT CAA GAC CTG CTG GAT GC-3' (McLeish M). The 16S (ribosomal RNA) gene fragment was amplified using *CI-J-2195* 5'-CCG GTC TGA ACT CAG ATC ACG T-3' (Simon *et al*., 1994) with *A2735* 5'-CGC CTG TTT AAC AAA AAC AT-3' (Crespi B). We amplified up to 1245bp of the COI mitochondrial gene, 444bp of the EF-1α gene, 472bp of the 16S, and 549 of the *wingless* gene. SeqEd v1.0.3 (http://helix.nih.gov/docs/gcg/seqed.html) was used to edit sequences. Sequences were aligned using CustalX 1.81.1a software (Thompson *et al*., 1997; ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/ accessed 24 June 2005). All nucleic acid sequence data has been lodged with GenBank under the accession numbers AY827474-AY827481, AY920988-AY921000, AY921058-AY921069, and DQ246453-DQ246516.

Taxon sampling is known to effect the estimation of substitution parameters. Our data set of 57 taxa was in excess of the suggested 20, assumed to be appropriate in accounting for uncertainty caused by too small a sample (Sullivan *et al*., 1999). In 5 instances, the dataset includes replicate populations of the same 'host race' from samples taken at either different sites or different

years. These taxa include populations of *K. sterni*, *K. waterhousei* (on *A. papyrocarpa*), *K. intermedius*, and *K. rugosus* (on *A. cana* and x2 races on *A. papyrocarpa*). We included these populations to verify expectations of phylogenetic coherence within a taxon.

Phylogenetic analysis

Phylogenies were inferred to validate the independence of the *K. rugosus* and *K. waterhousei* groups and extend and revise the current gall-inducing thrips phylogeny. The current robust well-resolved and well-supported gall-thrips phylogeny (Morris *et al*., 2001) comprises 21 described species and was generated using maximum parsimony (MP) and maximum likelihood approaches. We infer MP phylogenies with the addition of 32 new taxa using the search parameters consistent with Morris *et al*. (2001). To accommodate differences in substitution rate parameters and base compositional bias in our multiple gene dataset we implemented a model-based maximum likelihood Bayesian approach (see Appendix II). A recent study (Lin & Danforth, 2004) advocates a Bayesian approach to accommodate substitution and rate dynamics evident in the gall-thrips sequence dataset of Morris *et al*. (2001). We also conducted Bayesian analyses using a combined dataset (i.e. no partitioning of the sequence data) in addition to analyses using separated data (see Appendix III). In all cases, nodal support for poorly and well-supported relationships were invariably reproduced by the combined Bayesian analyses.

Maximum parsimony and Bayesian inferences were implemented in PAUP*b4.10 (Swofford, 2002) and MrBayes (MrBayes 3.0b4, Huelsenbeck and Ronquist, 2001) respectively. Maximum parsimony analysis was implemented using a heuristic search, with TBR (tree bisection-reconstruction), branch swapping on all best trees, with 100 random sequence additions holding 10 trees held at each step. We used 500 heuristic search pseudoreplicates to calculate bootstrap support values (Felsenstein, 1985), using the same search parameters as above for the pseudoreplicates. Heuristic search starting trees for branch-swapping were generated using stepwise addition, swapping on the best trees only.

To accommodate differences in substitution rate parameters and heterogeneity of base composition in our multiple gene fragment dataset we fitted separate models to different gene partitions in the Bayesian analysis (Lin & Danforth, 2004). The sequence data used in the MrBayes analysis was divided into six partitions comprising 1st, 2nd, and 3rd codon positions of the *cytochrome oxidase one* (COI) mitochondrial data, with single partitions for each of *elongation factor one alpha* (EF-1α), *wingless*, and the 16S gene fragments. We used a general time reversible (GTR) DNA substitution model with gamma distributed rates with a proportion of invariant sites. Posterior probabilities and mean branch lengths are derived from 3000 trees taken from generations 3.5-5.0 million, sampling every 500th generation. The sampled trees were derived from post-burnin generations after the chains had reached apparent stationarity. We ran the Bayesian analysis 3 times to verify the repeatability of the phylogenetic outcome. We summarised the repeatability between independent Bayesian runs by percent variation of the log likelihood arithmetic means generated for all generations sampled and for the post burnin sample trees. Log likelihood values reached apparent stationarity rapidly in each of the 3 Bayesian analyses. The arithmetic mean of the log likelihood values for all generations sampled and for post burnin samples were calculated for each Bayesian analysis and the percent variation between them determined. Percent variation between the arithmetic means between successive Bayesian analyses was only 0.11 percent.

The outgroup, *Rhopalothripoides* (Bagnall) and *Dactylothrips* (Bagnall), are the most closely related sister-genera to *Kladothrips* Froggatt (Morris *et al.*, 2002). The *Kladothrips rugosus* and *Kladothrips waterhousei* species complexes were chosen as ingroup taxa as these groups are expected to have diversified recently and in parallel on the same set of host *Acacia* species.

Codivergence analysis

Conservative associations between two gall-inducing thrips groups inhabiting the same *Acacia* host species suggest that the groups have diversified in parallel (Crespi *et al*., 2004). A comprehensive host-plant phylogeny is not available to compare with a gall-thrips phylogeny. Under a hypothesis of

parallel diversification, both groups are expected to respond to host speciation in tandem. To address the hypothesis of parallel divergence, congruence between two gall-thrips phylogenies inhabiting the same host species was tested. We inferred separate phylogenies of the *K. rugosus*, *Kladothrips acaciae*, and *Kladothrips ellobus* group and the *K. waterhousei*, *K. habrus*, and *Kladothrips hamiltoni* group using a Bayesian approach sampling every 500th of 3 million generations. Stationarity was reached almost instantaneously in our phylogenetic inferences and to reduce computational time, we sampled every 3 million generations when generating the trees used to test codivergence hypotheses. The data was analysed using a combined approach in addition to analysing partitioned data using the same priors as described above. Nodal support differences between the separate and combined approaches were negligible, and have therefore elected to show inferences generated using the partitioned analyses. Nearest ancestral sister-species were used as outgroups and pruned for the cospeciation analyses. We assumed that both of the inferences were 'true phylogenies.' As codivergence analyses assume that the phylogenies used represent the true relationships among taxa, the trees are not collapsed and support values are given for all bifurcations to demonstrate regions of uncertainty.

The extent to which host and parasite phylogenies are congruent can be used to detect 'coevolution' or cospeciation. To test how closely the diversification of gall-thrips has been subject to host speciation we tested for concordance between the phylogenies of: I) the *K. acaciae*, *K. ellobus*, and the *K. rugosus* complex; and ii) the *K. harpophyllae*, *K. hamiltoni*, *K. habrus, K. intermedius*, and *K. waterhousei* complex. Three approaches using computer programs were used, each treating the data different ways. First, ParaFit (Legendre *et al*., 2002) http://www.bio.umontreal.ca/casgrain/en/labo/parafit.html) was used to test a global null hypothesis that the association of two trees has been independent. This approach permits the treatment of the phylogenies 'symmetrically' and is not directed at reconstructing a putative history of the association. The associations between the phylogenies are randomised and tested. Phylogenies of the *K. rugosus* and *K. waterhousei* races are transformed into matrices of

principle coordinates and then combined with another matrix describing the associations between the phylogenies. The significance of a global fit is tested without direct inclusion of either one of the phylogenies rather focusing on manipulating the associations. To test the global fit between the *K. rugosus* and *K. waterhousei* groups, we implemented ParaFit using phylogenies of equal length branches; patristic distances generated in PAUP, and likelihood values from our Bayesian consensus phylogram. By using equal branch lengths (of 1) we were able to test the fit of the topologies only. Cospeciation predicts that codivergences must occur in a contemporaneous manner. The matrices approach also allows phylogenies to be represented by likelihood or patristic values that account for concordance subject to branch length variation.

Finally, as the statistical power afforded by matrix-driven approaches, such as ParaFit, was considered less than optimal as a result of information loss, concordance between *K. rugosus* and *K. waterhousei* phylogenies was also assessed using randomisation tests implemented in the event-driven TreeMap 1.0 (http://www.evolve.zoo.ox.ac.uk/rod/treemap.html; Page, 1994) and TreeFitter (http://www.ebc.uu.se/systzoo/research/treefitter/treefitter.html; Ronquist, 1997) approaches. Tree fitting and tree mapping methods are 'asymmetrical' in their treatment of the two trees to be tested for congruence. TreeMap (Page, 1994) and TreeFitter (Ronquist, 1997) are event-based comparisons that enable the maximisation of codivergence events (equal to 1) explaining the association between the two phylogenies by down-weighting duplication, sorting, and switching events (equal to 0 in each case). These events are of course invalidated when comparing parasite phylogenies that presumably do not have historic host-parasite associations. The difference between these two approaches is that TreeMap requires event costs to be treated in a more inflexible manner where the codivergence cost is strictly less than that of a duplication. TreeFitter allows a range of cost events to be explored where both duplication and codivergence can be set to zero. The ability to consider a greater range of event cost combinations in a single analysis can be important for understanding the optimal set of events more likely to represent the mechanisms operating in any given phylogenetic association. Here, we find both approaches less than ideal for our 'parasite-

parasite' association. However, we considered this type of approach a useful alternative to exploring the data and used each of the gall-thrips phylogenies as a pseudo-host tree in separate analyses.

To easily visualise the host associations among the *K. rugosus* and *K. waterhousei* species complex a tanglegram was generated by inferring Bayesian majority rule consensus phylogenies for each group and connecting taxa associated with the same host *Acacia* species. These trees were used to generate a set of possible codivergence events as inferred by TreeMap 1.0. TreeMap provides a graphic utility that generates a tanglegram displaying the associations and putative codivergence events between two phylogenies. The significance of the association between phylogenies is determined between observed and randomised trees generated from either of the phylogenies being compared. An approach that randomises a host phylogeny is intuitive for host-parasite associations but not optimal when neither tree is a 'host' as such. Here, we assume that either of the phylogenies is likely to closely match that of the true host phylogeny under cospeciation criteria, where either of the thrips phylogenies can be used to simulate the host phylogeny.

RESULTS

We extended and revised the phylogeny of Morris *et al.* (2001) with the inclusion of 16 putative races (on different *Acacia* species) of the *K. rugosus* complex and 10 putative races of the *K. waterhousei* complex that specialise on the same 14 host *Acacia* species. Maximum parsimony and Bayesian inferences are in general agreement and show a high level of support for each of the clades containing the *K. rugosus* and *K. waterhousei* complexes (Figure 1-2). Phylogenetic concordance tests between the *K. rugosus* and *K. waterhousei* species complexes showed a significant level of non-independence.

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Phylogenetic analysis

The addition of 32 new taxa in our phylogenetic inferences yielded results consistent with the general structuring of the most recent published phylogeny (Morris *et al*., 2001). Both maximum parsimony and Bayesian inferences are in general agreement and indicate that the *K. rugosus* and *K. waterhousei* clades form well supported and well resolved groups of 100% for each of these nodes in the Bayesian inference and 99% in the MP inference (Figures 1-2). A majority of the putative *K. rugosus* and *K. waterhousei* host races appear to be at various stages of differentiation (Figure 3). In particular, taxa exhibiting apparent less than species level genetic distances with similar gall structures group as clades. Uncorrected "p" distances for COI (see Appendix IV) between *K. rugosus* population bearing disparate gall structures were in the order of 6% - 10% contrasting those among like types with distances not exceeding 0.8%. Uncorrected "p" distances between the outgroup and *Kladothrips* species ranged from 7% to 12%. Both MP and Bayesian inferences grouped 'pouched,' 'elongate,' and 'spiky' gall structures into clades of like types. Replicate taxon samples of populations with the same host and gall structure collected from different sites or seasons grouped as sister-taxa. These grouping confirmed the expectation that such populations, particularly at relatively recent stages of differentiation, maintained phylogenetic coherence. For example, although currently considered host races, populations of *K. waterhousei* inhabiting *Acacia cana* and *A. papyrocarpa* sampled across different years and multiple sites, group together.

The *K. rugosus* complex was paraphyletic with respect to *Kladothrips maslini* and the *K. waterhousei* complex was paraphyletic with regard to the putative host races of *K. habrus* and *K. intermedius*. The Maximum Parsimony inference places *Kladothrips rodwayi* into a paraphyletic relationship with the *K. waterhousei* complex. We suspect low bootstrap support and/or long-branch attraction, due to incomplete sequence data (see Wiens, 2005 for explanation) for the *K. waterhousei* complex at EF-1a and *wingless* gene regions, might have contributed to this outcome. The clade comprising *K. acaciae*, *K. ellobus*, and the *K. rugosus* complex, matches closely with host affiliation by the sister-clade comprising *K. harpophyllae*, *K. hamiltoni*, and the *K. waterhousei*

complex. Sister-species from each of these clades specialise on the same host species, *Acacia cambadgei* (Baker) and *Acacia harpophylla* (Muell, Benth) with the *K. rugosus* and *K waterhousei* complexes sharing the same set of host species. Monophyly of the *K. rugosus* complex becomes invalid by the presence of the *K. maslini* lineage, though a host shift is strongly suspected along this lineage (Crespi *et al*., 2004).

Codivergence analysis

Analyses of cospeciation were inferred indirectly by testing concordance between the phylogenies of two thrips clades that share the same host species. Therefore, inferences of cospeciation are derived under the assumption that non-independence between the two parasite phylogenies suggests these were subject to extrinsic factors (host divergence) that resulted in parallel divergence events. ParaFit tests a global null hypothesis that the association between the phylogenies has been independent ($P = 0.01$). Tests on individual associations indicate that this inferred cospeciation was partial rather than complete. Portions of the two trees are apparently independent as 3 of the 12 links were significant in their contribution to the global test statistic. Global tests using patristic distances and likelihood values were non-significant ($P = 0.08$ and $P = 0.18$ respectively), suggesting that non-contemporaneous associations invalidated the significant codivergence events or that molecular-evolutionary rates differ between lineages in the two clades.

Under the assumption that one of the thrips phylogenies was equal to the true host phylogeny, both thrips phylogenies were used to simulate the host phylogeny in separate analyses in TreeMap 1.0 and TreeFitter. An exact search algorithm implemented in TreeMap 1.0 produced 24 solutions explaining the historical relationship between the phylogenies. All required 7 codivergence events, when the *K. rugosus* group was assumed to represent the true host phylogeny. One such solution is summarised in Figure 4 and it also shows gall-thrips taxa that share the same host species. An exact search generated 19 solutions all incorporating 7 codivergence events when the *K. waterhousei* group was assumed to represent the true host phylogeny. Both trees were randomised using a Markovian model to generate a distribution ($n = 10000$) of

codivergence events. The results indicated a significant ($t \leq t_{0.05(1),\infty}$, reject H_o of no difference) association between the trees. This outcome indicates that cospeciation apparently occurred more often than by chance. A randomisation approach implemented in TreeFitter was used to test the significance of codivergences in the observed tree associations in two separate analyses, where alternate phylogenies were assumed to match the true host phylogeny. Tests of congruence against a randomised set of trees indicated a significant association (where host tree is *K. waterhousei* group; $P = 0.019$ and where host tree is *K. rugosus* group; $P = 0.016$).

DISCUSSION

We inferred phylogenies including numerous undescribed putative species of gall-inducing thrips to test hypotheses of diversification. The addition of these taxa to a previous gall-thrips phylogeny (Morris *et al*., 2001), results in a tree that includes virtually all known taxonomic entities for this group. A clear pattern has emerged from this expanded tree, suggesting that diversity for this group of specialist insects is generated in close association with host speciation. Maximum parsimony and Bayesian inferences group the *K. rugosus* and *K. waterhousei* species complexes as closely related groups with a high level of support (Figures 1 & 2). Significant congruence between the phylogenies of the *K. rugosus* and *K. waterhousei* groups, including sister-species within each (Figure 4), indicated both cospeciation and convergence of thrips across host-plant species of Acacia in the section *Plurinerves*.

Convergence of thrips lineages among related hosts would predict their phylogenies to be independent of one another. Deviations from an identical match would be indicative of processes other than cospeciation operating. The *K. rugosus* and *K. waterhousei* groups might have independently converged onto the same set of closely related *Acacia* conducive to *Kladothrips* gall-induction, as host shifts are reported (Craig *et al*., 1994) to more freely occur among taxonomically and phylogenetically similar plants. Contradicting the model involving complete cospeciation is evidence that indicates the apparent

coincidence in several species of major morphological and life history changes accompanying host switches between more distantly related host lineages that are not inhabited by closely related thrips sister-species (Crespi *et al*., 2004). These switches are also evidenced by the absence of elongate-pouched gall type combinations and by the presence of only a single gall type on the novel host species, as in *Kladothrips intermedius*, *Kladothrips rodwayi* and *Kladothrips morrisi* (Crespi *et al*., 2004).

Codivergence between these thrips groups indicates they were subject to the same isolating events imposed by host speciation. Poor resolution among recently diverged taxa within the two complexes renders cospeciation inferences ambiguous for some associations. Host switching and the formation of host races among closely related species of this *Acacia* section does not appear to be accompanied by the relatively large shifts in adaptive change when switching between host sections (Crespi *et al*., 2004) as implied for *K. maslini* and *K. rodwayi*. Highly similar morphologies and marginal adaptive shifts among thrips belonging to the *K. rugosus* complex belie substantial genetic differentiation evident in the COI gene and the structures of the galls induced by each. This work suggests that the potential of specialist phytophagous insects to diversify phenotypically increases with their ability to colonise more distantly related hosts.

Phylogenetic analyses

Phylogenetic inferences reveal patterns consistent with some taxa belonging to the *K. rugosus* and *K. waterhousei* complexes being genetically differentiated below the level evident among the described gall-thrips taxa (Morris *et al*., 2001, Crespi *et al*., 2004, McLeish *et al*., 2006). These recently diverged groups offer an opportunity to test codivergence hypotheses where evolutionary processes such as extinction are unlikely to obscure interpretation, as might be expected amongst relatively older lineages (Brown *et al*., 1995).

The morphological difference between the *K. intermedius* samples with ‘pouched’ and ‘elongate’ gall types, was considered negligible (personal communication, Mound LA) though genetic differentiation of 5% (uncorrected “p” distance for COI) suggests considerable differentiation. The *K. sterni*

populations from Western Australia (WA) and Queensland (QLD) grouped as sister-taxa with a high level of support (Figures 1-2) although branch length estimates (Figure 4) indicate genetic divergences consistent with the geographic distance between them. The grouping of *K. habrus* with putative *K. intermedius* populations requires further attention to establish taxonomic boundaries and nomenclature. Indeed, lack of phylogenetic signal evident for taxa within these groups compounds tests of phylogenetic concordance due to considerable levels of uncertainty (Figure 4). However, this ambiguity does not invalidate a signal that was strong enough to produce a significant level of non-independence in tests of congruence between these parallel clades affiliated with the same host species.

Diversification

It has been hypothesized that cospeciation and host switching processes are responsible for the genetic, phenotypic, and ecological differentiation in gall-thrips (Crespi *et al*., 2004). Strong agreement among phylogenetic inferences indicates that the *K. rugosus* and *K. waterhousei* complexes are both paraphyletic and inhabit *Plurinerves* host species (Figures 1-2). These two thrips clades might have diversified in parallel via each shifting to related, nearby hosts without cospeciation *per se*, but broad patterns of gall-thrips lineages tracking host diversification and evidence of parallel diversification between thrips and host *species* at lower scales appears to be partially an outcome of cospeciation.

The best available *Acacia* host phylogeny (Crespi *et al*., 2004) provides some support for the relationships indicated by the *K. rugosus* and *K. waterhousei* groups and associated sister-species (Figure 4). *A. harpophylla* and *Acacia cambagei* (Baker) are sister taxa and are hosts to basal sister species of both thrips clades. Although the phylogenetic relationships among the other *Plurinerves* species are weakly supported, this group forms a sister clade to *A. harpophyllae* and *A. cambagei*. The tanglegram in Figure 4 predicts that *A. cambagei* and *A. harpophyllae* are a sister-clade to hosts that include species that have undergone a recent radiation. Switching between host plants more distantly related than hosts of gall-thrips sister-taxa, is

accompanied by noticeable life history shifts, is very likely to occur between hosts that are taxonomically and phylogenetically close, and have overlapping or adjacent ranges (Maslin, 2001; Crespi *et al*., 2004). Several examples stand out. Within the clade also comprising the *K. waterhousei* group, losses in sociality (Morris *et al*., 2001) accompanied by an apparent switch to more distantly related host have been inferred (Crespi *et al*., 2004). *Kladothrips xiphius* is believed to represent a loss in sociality and is found on a species belonging to the *Juliflorae* section, not *Plurinerves* as do sister-species. Similarly, *K. rodwayi* is recognised as a loss in sociality and it too is found on a more distantly related host, *A. melanoxylon* (Figure 4), a species distributed in temperate and not arid climates. *Kladothrips intermedius* inhabits a host species that appears not to be as closely related to the hosts of thrips sister-species. Like *K. rodwayi*, *K. intermedius* ecloses within the gall, contrasting other members of this thrips clade that instead disperse as pupa. Unlike its *K. rugosus* sister-members on a *Plurinerves* host and disperse as pupa, *K. maslini* inhabits a *Juliflorae* host (Figures 1 & 2) and ecloses as an adult within the gall. Uncorrected "p" distance (unpublished work) variation shown by these additional taxa implies both similar and below those between described gall-thrips species.

The frequency and causes of those host switches traversing host sections accompanying relatively large life history changes is unknown. A period of host radiation may contribute to gall-thrips ability to switch among host-plants under speciation. It appears that the opportunity for gall-thrips to diversify might have been assisted by speciation in *Plurinerves* hosts presumably during a rapid radiation as widespread aridity developed in the early Quaternary (Maslin & Hopper, 1982; Clapperton, 1990; Lovejoy & Hannah, 2004). It is expected that gall-inducing insects should radiate into arid habitats as pressures exerted by parasitoids, predators, and pathogens are not as acute in xeric environments (Fernandes *et al*., 1994; Blanche & Westoby, 1995; Price *et al*., 1998; Veldtman & McGeoch, 2003). Radiations into novel niches to relieve pressures exerted by natural enemies is consistent with Ehrlich and Raven's (1964) 'escape and radiate' hypothesis predicting the generation of diversity in phytophagous insects as a consequence of strong

selection after colonising a novel host. However, reduced mortality imposed by natural enemies is dependent on the presence/absence of predators and parasitoids associated with the novel host that has been colonised (Gratton & Welter, 1999; Schönrogge *et al*., 2000; Mira & Bernays, 2002). Although the role such tri-trophic interactions play in diversification of phytophagous insects have been recognised for some time, attempts to reconcile these types of associations within a community structure framework are not well represented in the literature (Singer & Stireman, 2005).

Bimodality in divergence

The tanglegram in Figure 4 shows phylogenies of both the *K. rugosus* and *K. waterhousei* complexes connected by lines that join terminal taxa that share the same host species. One of many possible inferred solutions explaining codivergence events between the trees is indicated at particular bifurcations. Tests for concordance indicate that these phylogenies are significantly non-independent, but a notable degree of incongruence is also evident. However, phylogenetic uncertainty in the trees might contribute to a component of ambiguity in the interpretation of codivergence events possibly caused by inclusion of taxa in the phylogenies that are marginally divergent. Synchronous genetic isolation between host and parasite can occur at the level of individual, population, species, or higher (Rannala & Michalakis, 2003). Codivergence indicates a frequent incidence of isolating events affecting both thrips clades in tandem and that cospeciation between gall-thrips and *Acacia* operates in a broad sense between lineages and also between species. A lack of identical fit between the phylogenies shows independent isolating events has possibly acted on either clade at some stage. Non-significant contemporaneous branching episodes between the *K. rugosus* and *K. waterhousei* groups shown by the ParaFit outcomes for patristic and likelihood branch length values suggest bimodality in divergence processes. That is, each thrips clade appears to have responded somewhat differentially to isolating events of the host. Given cospeciation, one might expect that the more closely related host species would reflect proportionally similar divergences paralleled in both thrips lineages that

inhabit these hosts, but this pattern does not appear to be consistent with our data.

Bimodality in divergences might reflect differential host-utilisation, dispersal ability, or community driven differences such as escaping natural enemies and interspecific competition (Jaenike, 1990). For example, demographic and life history differences between the *K. rugosus* and *K. waterhousei* complexes, such as social organization (Crespi, 1992) and comparative brood sizes (Wills *et al*., 2001) could be invoked to develop hypotheses explaining this bimodality. There is general agreement between gall-thrips and best available host phylogenies (Crespi *et al*., 2004) but interpretations are also conditional on the presence of a degree of phylogenetic uncertainty. The ability to test concordance between gall-thrips lineages has provided insight into mechanisms driving diversification in this group where comparisons of insect and plant phylogenies were not feasible. This work shows that diversification in phytophagous insects can proceed via a combination of synchronous divergence episodes between insect and host lineages in addition to independent modes of speciation among them. Speciation by host switching and host race formation, concurrent with cospeciation, can play a role in generating diversity. The potential for a group of specialist phytophages to diversify appears to be closely linked to their ability to traverse genetic, phenological, chemical, and morphological obstacles among plant species varying in susceptibility to colonisation. These patterns imply that the ability to overcome difficult barriers to colonising novel host plants might determine the degree of diversity attained in phytophagous insects. Switching to more distantly related hosts should be accompanied by greater potentials to diversify.

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Table 1: Description and locations of collection sites for the *Kladothrips rugosus* and *K. waterhousei* species complexes used for the sequence data. For collections details of other species see Morris *et al*. (2001). Host populations of *K. habrus, K. intermedius*, and *K. waterhousei* were also collected.

Galler	Host	Site description	Date
K. sterni	*A. aneura*	20 km N of Quilpi QLD	Aug 04
K. habrus	*A. pendula*	15 km S of Yenda NSW	May 04
K. intermedius	*A. oswaldii*	15 km W of Mt. Hopeless SA	Mar 04
K. rugosus	*A. papyrocarpa*	25km W of Whyalla SA	Feb 02
K. rugosus	*A. ammophila*	Lk. Bindegolly QLD	Apr 97
K. rugosus	*A. ancistrophylla*	20 km E of Dalwallinu WA	Apr 97
K. rugosus	*A. enervia*	3 km W of Merridin WA	Jan 99
K. rugosus	*A. cana*	25 km NW of Tibooburra NSW	Apr 98
K. rugosus	*A. loderi*	83 km SW Broken Hill NSW	Apr 05
K. rugosus	*A. melvillei*	9 km S of Yenda NSW	May 04
K. rugosus	*A. microcephala*	10 km W of Prairie QLD	Apr 98
K. rugosus	*A. microsperma*	40 km E of Quilpie QLD	Aug 04
K. rugosus	*A. omalophylla*	19 km S of Miles QLD	Apr 98
K. rugosus	*A. papyrocarpa*	25km W of Whyalla SA	Feb 02
K. rugosus	*A. pendula*	95 km S of Griffith NSW	Apr 04
K. rugosus	*A. sibilans*	9 km S of Wooramel WA	Apr 97
K. rugosus	*A. tephrina*	8 km N of Barcaldine QLD	Feb 04
K. waterhousei	*A. maranoensis*	57 km E of Morven QLD	Apr 97
K. waterhousei	*A. loderi*	79 km W of Wilcannia NSW	Mar 96
K. waterhousei	*A. ancistrophylla*	20 km E of Dalwallinu WA	Apr 97
K. waterhousei	*A. enervia*	3 km W of Merridin WA	Jan 99
K. waterhousei	*A. cana*	25 km NW of Tibooburra NSW	Apr 98
K. waterhousei	*A. microsperma*	45 km N of Adavale QLD	Apr 97
K. waterhousei	*A. omalophylla*	121 km E Quilpie QLD	Apr 98
K. waterhousei	*A. ammophila*	Lk. Bindegolly QLD	Apr 97
K. waterhousei	*A. microcephala*	37 km N of Aramac QLD	Mar 98

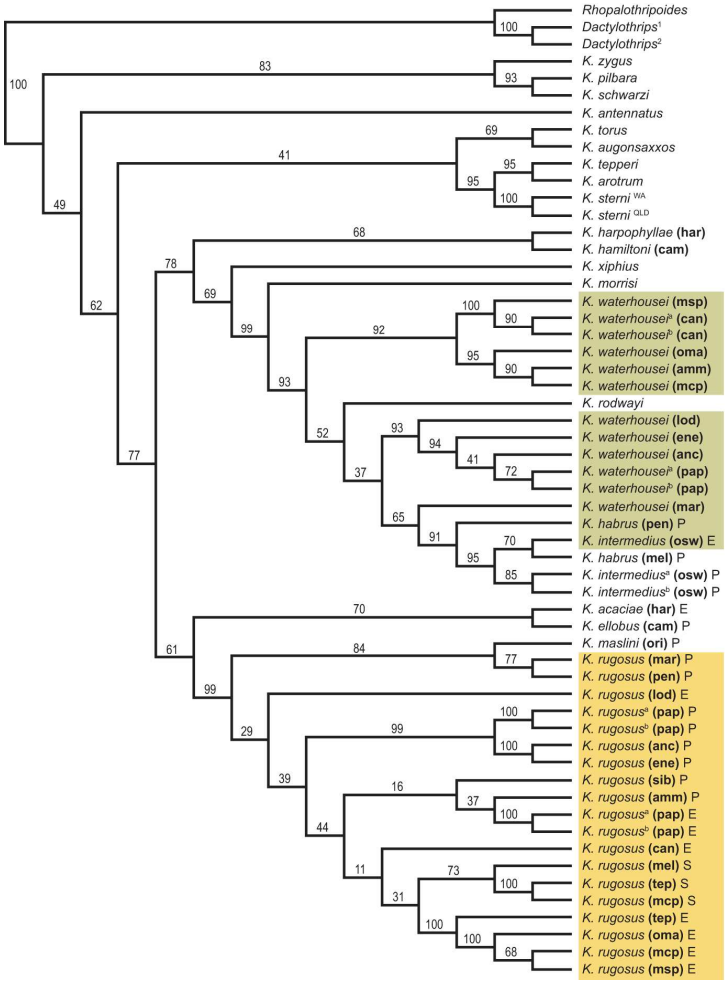


Figure 1: A maximum parsimony phylogeny using COI, EF-1α, *wingless*, and 16S genes. A heuristic search with tree bisection-reconstruction (TBR) branchswapping, random addition of taxa 100 replicates per search and 10 trees held at each step), and 500 bootstrap replicates was implemented. The coloured boxes indicate the *K. rugosus* and *K. waterhousei* species complexes. Host tree species are abbreviated in brackets as follows: *A. ammophila* (amm), *A. ancistrophylla* (anc), *A. cana* (can), *A. enervia* (ene), *A. loderi* (lod), *A. maranoensis* (mar), *A. melvillei* (mel), *A. microcephala* (mcp), *A. microsperma* (msp), *A. omalophylla* (oma), *A. oswaldii* (osw), *A. papyrocarpa* (pap), *A. pendula* (pen), *A. sibilans* (sib), and *A. tephrina* (tep). Taxon codes are as follows: QLD = Queensland and WA = Western Australia populations, a = type A, b = type B, E = elongate, P = pouched, and S = spiky gall structures.

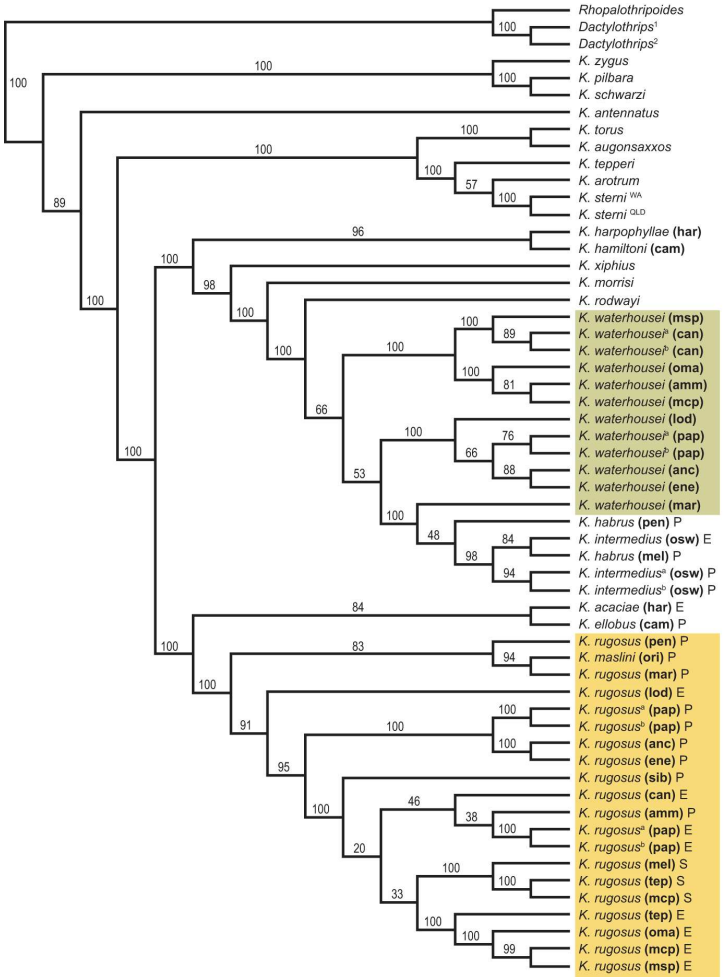


Figure 2: Bayesian Consensus tree analysis of six separately modelled partitions comprising 1st, 2nd, and 3rd COI codons and separate EF-1α, *wingless*, and 16S sites. Posterior probabilities and mean branch lengths are derived from 3000 trees taken from a sample of 5 million generations, sampling every 500th generation. The coloured boxes indicate the *K. rugosus* and *K. waterhousei* species complexes. Host tree species are abbreviated in brackets as follows: *A. ammophila* (amm), *A. ancistrophylla* (anc), *A. cana* (can), *A. enervia* (ene), *A. loderi* (lod), *A. maranoensis* (mar), *A. melvillei* (mel), *A. microcephala* (mcp), *A. microsperma* (msp), *A. omalophylla* (oma), *A. oswaldii* (osw), *A. papyrocarpa* (pap), *A. pendula* (pen), *A. sibilans* (sib), and *A. tephrina* (tep). Taxon codes are as follows: QLD = Queensland and WA = Western Australia populations, a = type A, b = type B, E = elongate, P = pouched, and S = spiky gall structures.

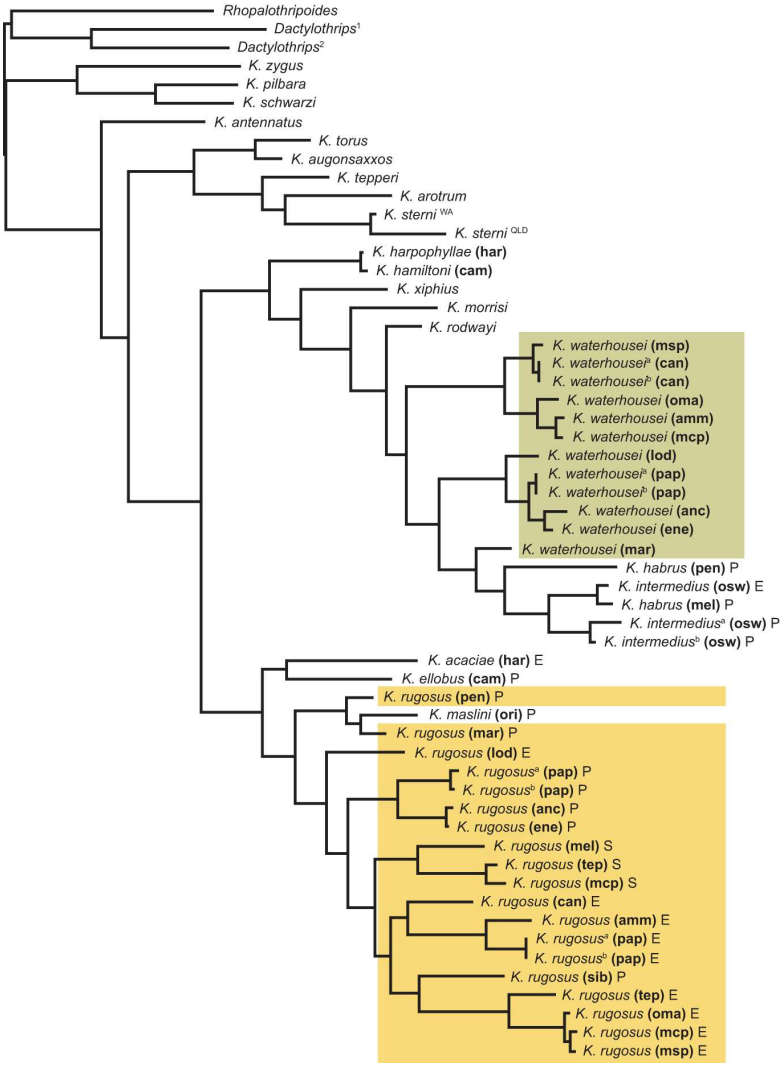


Figure 3: Consensus phylogram using six separately modelled partitions comprising 1st, 2nd, and 3rd COI codons and separate EF-1α, *wingless*, and 16S sites. The coloured boxes indicate the *K. rugosus* and *K. waterhousei* species complexes. Host tree species are abbreviated in brackets as follows: *A. ammophila* (amm), *A. ancistrophylla* (anc), *A. cana* (can), *A. enervia* (ene), *A. loderi* (lod), *A. maranoensis* (mar), *A. melvillei* (mel), *A. microcephala* (mcp), *A. microsperma* (msp), *A. omalophylla* (oma), *A. oswaldii* (osw), *A. papyrocarpa* (pap), *A. pendula* (pen), *A. sibilans* (sib), and *A. tephrina* (tep). Taxon codes are as follows: QLD = Queensland and WA = Western Australia populations, a = type A, b = type B, E = elongate, P = pouched, and S = spiky gall structures.

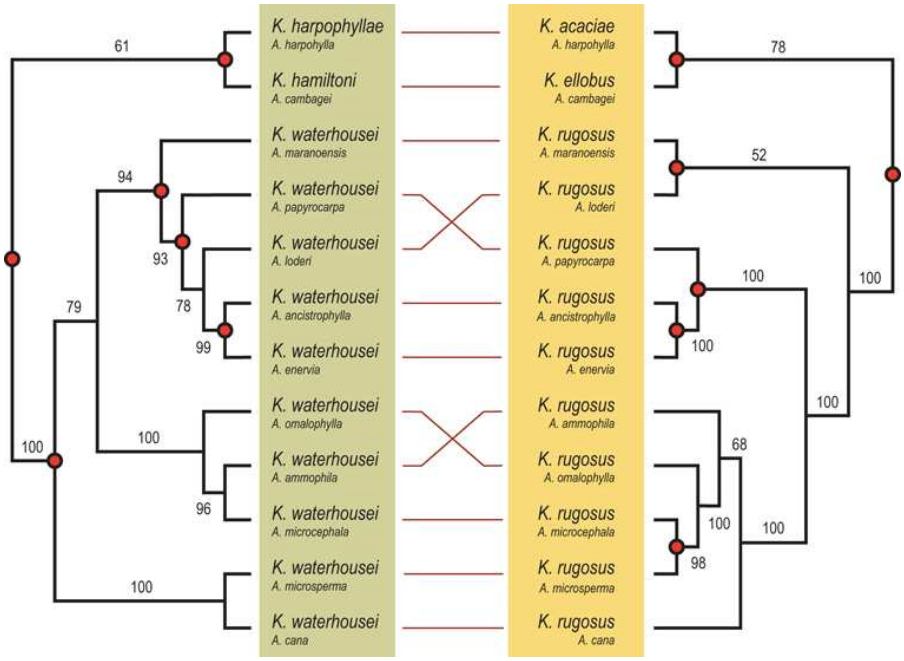
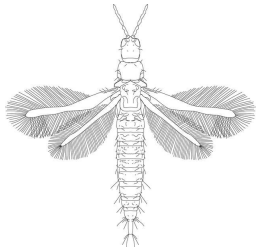


Figure 4: Bayesian Consensus phylogenies of the *K. rugosus* (gold box) and *K. waterhousei* (green box) groups plus associatied host species. Posterior probabilities are indicated on branches. Host species are indicated under thrips taxon names. Lines connected to two phylogenies show host associations. Circles on nodes show codivergence events as inferred by TreeMap 1.0.



CHAPTER III.

HOST-DRIVEN DIVERSIFICATION OF GALL-INDUCING *ACACIA* THRIPS

A version of this chapter has been published as:

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ABSTRACT

Insects that feed on plants contribute greatly to the generation of biodiversity. Hypotheses explaining rate increases in phytophagous insect diversification and mechanisms driving speciation in such specialists remain vexing after considerable attention. Proliferation of plant-feeding insects and their hosts are expected to broadly parallel one another. Climate change over geological timescales imposes consequences for the diversification of flora and fauna via habitat modification. This work uses a phylogenetic approach to investigate the premise that the aridification of Australia and subsequent expansion and modification of arid-adapted host flora has implications for the diversification of insects that specialise on them. Likelihood ratio tests indicated the possibility of hard molecular polytomies within two co-radiating gall-inducing species complexes specializing on the same set of host species. Significant tree asymmetry is indicated at a branch adjacent to an inferred transition to a *Plurinerves* ancestral species. Lineage by time diversification plots indicate gall-thrips that specialise on *Plurinerves* hosts differentially experienced an explosive period of speciation contemporaneous with climatic cycling during the Quaternary. Chronological analyses indicated that the approximate age of the origin of gall-inducing thrips on *Acacia* might be as recent as 10 million years ago during the Miocene as truly arid landscapes first appeared in Australia. Nested clade analyses for one lineage indicated historical population fragmentation and restricted gene flow among gall-thrips populations expected under the Quaternary climatic cycling hypotheses. Host-plant diversification and spatial heterogeneity of hosts have increased the potential for specialisation, resource partitioning and unoccupied ecological niche availability for gall-thrips on Australian Acacia.

INTRODUCTION

Determining the driving forces behind the diversification of phytophagous insects remains a central challenge to biology. Increases in insect species numbers can be expressed as an outcome of their feeding on plants (Ehrlich & Raven, 1964). The evolution of this type of ecological relationship between specialists and patterns of host plant affiliation has been well documented (Futuyma & Moreno, 1988; Jermy, 1993; Farrell & Mitter, 1998; Ronquist, 1998; Kelley *et al*., 2000; Craig *et al*., 2001; Nyman, 2002; Johnson & Clayton, 2003; Page, 2003; Ward *et al*., 2003). The majority of phytophagous insect species are specifically associated with and generally show preference for single plant families presumed to be evolutionarily conservative. This is displayed in narrow food specialisation and life habits for the majority of insect species. The mechanisms responsible for the generation of diversity in plant-insect systems are usually thought to result from strict cospeciation or frequent host shifts followed by specialisation. Host range expansion is believed to provide opportunities for vicariance, and the subsequent effects of selection or drift, fuel the diversification of specialists (Janz *et al*., 2006). This is in line with predictions that reciprocal radiations should occur for some insect-plant associations (Cranston & Naumann, 1991; Farrell & Mitter, 1998; Gaston, 1998) where the proliferation and distribution of endoparasites and their hosts are expected to emulate one another.

The evolution of endemic biodiversity in contemporary Australia has primarily arisen over a period of increasing aridity during the late Tertiary (Maslin & Hopper, 1982; Clapperton, 1990; Niklas, 1999; Markgraf *et al*., 1995; Lovejoy & Hannah, 2004). Truly arid landscapes appear some time after the Miocene (Wasson, 1982), and more recently, dramatic changes to the terrestrial environment occurred during the Quaternary when glacial-interglacial cycling drove a rapid increase in desertification. The aridification of Australia modified habitat enhancing the evolution of flora, including sclerophylly. Range expansion and contraction of sclerophylly during glacial cycling (Crisp *et al*., 2001; Burgman, 2002; Chapple & Keogh, 2004) would be expected to provide opportunity for explosive speciation (Hopper, 1979;

Maslin & Hopper, 1982; Truswell & Harris, 1982; Clapperton, 1990; Anderson, 1994; Markgraf *et al*., 1995; Crespi *et al*., 1998; Crespi & Worobey, 1998; Gaston, 1998; Price *et al*., 1998; Morris *et al*., 2002; Crisp *et al*., 2004). However, the proposal that faunal and floral diversification in Australia has been partly driven by climatic glacial-interglacial cycling effects over the last 2-3 million years remains a point of conjecture and difficult to test (Hopper & Gioia, 2004).

Global patterns of gall-inducing species richness are believed to increase from mesic to xeric environments across transitional vegetation gradients from mesophytic to sclerophyllous plants (Fernandes & Price, 1988; Fernandes *et al*., 1994; Blanche & Westoby, 1995; Price *et al*., 1998; Veldtman & McGeoch, 2003). Species-specific gall induction by thrips (Thysanoptera: Phlaeothripidae) on closely related desert *Acacia* (Leguminosae) trees has likely evolved as a consequence of the developing arid landscape (Ananthakrishnan, 1992; Crespi *et al*., 1998; Morris *et al*., 1999; Crespi *et al*., 2004). *Acacia* is a prominent endemic Australian xeromorphic plant (Boughton, 1986) and the most speciose plant genus on the continent, distributed throughout arid and semi-arid regions that cover over 70% of the Australian landmass (Anderson, 1994; Maslin *et al*., 2003). The presence of sclerophyllous phyllodes (modified petiole) is characteristic of *Acacia* on which gall-thrips induce galls (Mound, 1994) and are an adaptation to conserve water and to low nutrient environments (Atkin *et al*., 1998; Niklas, 1999; Miller *et al*., 2002; Doley, 2004). *Acacia* population subdivision is partly determined by soil type mosaics (Nelson, 1974; Parker *et al*., 1985; Haddad *et al*., 2001; Maslin, 2001) and this patchwork of nutrient poor sand dunes, sand plains, and rocky ranges provided the sclerophylly habitat and opportunity for speciation (Hopper, 1979; Maslin & Hopper, 1982; Hopper & Gioia, 2004). Patchy distributions of *Acacia* would contribute to heightened extinction and speciation rates (Cracraft, 1992). This type of environmental heterogeneity can potentially also provide a foundation for increased specialisation and resource partitioning (McKinney & Drake, 1998).

Sections *Plurinerves* and *Juliflorae* of the subgenus *Phyllodineae,* host to all but one gall-thrips species, do not appear to represent monophyletic groups but remain closely related to one another (Miller *et al*., 2003; Murphy *et al*., 2003). Species within these sections are believed to be relatively young compared to other sections in the subgenus *Phyllodineae* and more susceptible to speciation during the Pleistocene climatic cycling (Miller *et al*., 2003). Semi-arid transitional climatic zones on the periphery of the interior arid-zone have been proposed as having been particularly favourable for floral (Crisp *et al*., 2001; Crisp *et al*., 2004; Hopper & Gioia, 2004) and faunal (Christidis & Schodde, 1993; Chapple & Keogh, 2004; Strasburg & Kearney, 2005) speciation. The lack of sequence divergence within the chloroplast genome of host *Acacia* and there close relatives, as well as evidence of a rapid morphological radiation among *Acacia* in subgenus *Phyllodineae*, is symptomatic of a relatively young group (Miller *et al*., 2003). Speciation in the *Plurinerves* is believed to have occurred over a relatively short period as widespread aridity developed in the late Tertiary and early Quaternary (Maslin & Hopper, 1982; Clapperton, 1990) and that tropical section *Juliflorae* is believed to be more ancient than the *Plurinerves* section (Vassal, 1972).

Acacia thrips are a monophyletic group distinct from other gall-inducing groups in Australia (Morris *et al*., 2001) and have likely undergone diversification as a result of cospeciation, highly restricted host-plant shifting, or both processes (Chapter II). In this study we test the hypothesis that host specific gall-inducing thrips have diversified in close accordance with evolution of highly-related host *Acacia* in the subgenus *Phyllodineae* (DC.) Seringe, comprising more than 960 species largely confined to the Australian arid-zone (Maslin *et al*., 2003). Using a phylogenetic approach, we demonstrate that net increases in gall-thrips diversification are closely linked with host range expansion and aridification in Australia. We approach this study in three steps. First, to infer biogeographical processes acting on the genesis of diversity in gall-thrips/*Acacia* interactions, we make the assumption that gall-thrips diversification and host radiations are closely linked, and use species-level phylogenetic analyses and penalised likelihood approaches to date gall-thrips lineage diversification. Second, to address hypotheses of rate

increase in gall-thrips diversification, expected as an outcome of *Acacia* radiations, we use likelihood ratio tests to identify late-branching molecular polytomies correlated with periods of explosive radiation as predicted by Hopper and Gioia (2004), and tree asymmetry methods to identify rate changes. Third, to test hypotheses of past fragmentation and allopatry in gall-thrips populations of *Kladothrips nicolsoni* (McLeish, Chapman, & Mound) inhabiting a widespread host species, we use a nested clade approach and analysis of molecular variance (AMOVA) to test intraspecific genetic structure.

METHODS

Phylogenetic analysis

All sampled operational taxonomic units were included in the phylogenetic analyses comprising a great majority of known cryptic types and all described species (Morris *et al*., 2001). A comprehensive explanation of DNA extraction, PCR, and alignment protocols, and choices for substitution models, is given in Chapter II. Up to 1245bp of the *cytochrome oxidase one* (COI) mitochondrial gene, 444bp of the *elongation factor one alpha* (EF-1α) gene, 472bp of the 16S (ribosomal RNA subunit) gene, and 549 of the *wingless* gene were amplified. Sequence data for the *K. waterhousei* complex at EF-1α and *wingless* gene regions was not available and absent for all analyses. Sequences were aligned using CustalX 1.81.1sa software (Thompson *et al*., 1997; ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/ accessed 24 June 2005). Insertions and deletions were removed from the sequence alignment before analyses. All nucleic acid sequence data has been lodged with GenBank under the accession numbers AY827474-AY827481, AY920988-AY921000, AY921058-AY921069, and DQ246453-DQ246516. Maximum parsimony and Bayesian inferences were implemented in PAUP*b4.10 (Swofford, 2002) and MrBayes (MrBayes 3.0b4, Huelsenbeck & Ronquist, 2001) respectively. Maximum parsimony analysis was implemented using PAUP and we employed a heuristic search, with TBR (tree bisection-reconstruction), branch swapping on

all best trees, with 500 random sequence additions holding 10 trees held at each step. We used 1000 heuristic pseudoreplicates to calculate bootstrap support values (Felsenstein, 1985), using the same search parameters as above for the pseudoreplicates.

To accommodate differences in substitution rate parameters and heterogeneity of base composition in our multiple gene fragment dataset we fitted separate models to different gene partitions in the Bayesian analysis (Lin & Danforth, 2004; Brandley *et al*., 2005). The sequence data used in the MrBayes analysis was divided into six partitions comprising 1st, 2nd, and 3rd codon positions of the COI mitochondrial data, with single partitions for each of EF-1α, *wingless*, and the 16S gene fragments. We used a general time reversible (GTR) DNA substitution model with gamma distributed rates with a proportion of invariant sites. Posterior probabilities and mean branch lengths are derived from 3000 trees taken from generations 3.5-5.0 million, sampling every 500th generation. The sampled trees were derived from post-burnin generations after the chains had reached apparent stationarity. We ran the Bayesian analysis 3 times to verify the repeatability of the phylogenetic outcome using the same priors as above and obtained very consistent and stable posterior values. There was a 0.16% difference in the arithmetic mean of posterior probabilities between the 3 MrBayes runs.

The outgroup, *Rhopalothripoides* (Bagnall), is the most closely related sister-genus to *Kladothrips* Froggatt (Morris *et al*., 2002). The *Kladothrips rugosus* (Froggatt) and *Kladothrips waterhousei* (Mound & Crespi) comb. n. species complexes were chosen as ingroup taxa as these groups are believed to have diversified recently and in parallel (Chapter II). The approximation for the origin of each of these complexes is expected to be contemporaneous.

Construction of chronograms

The molecular clock hypothesis (Felsenstein, 1981) of equal rates across lineages can be examined using a likelihood-ratio test. The GTR + I + G model for all gene regions in a DNA sequence data set was assumed (Modeltest Version 3.06) in the analysis. The null hypothesis is that the likelihood is maximized under the constraint of equal rates across lineages (L_0), that is, an

enforced molecular clock model. The alternative hypothesis relaxes the clock constraint by assigning a different rate to each of the lineages; the likelihood under the alternative hypothesis is L_1. The likelihood-ratio test statistic (twice the difference in the $\log_e$ likelihood, $-2 \log_e \Lambda$; $\Lambda = L_0/L_1$) between the null and alternative models is approximately χ^2 distributed with s - 2 degrees of freedom. The molecular clock hypothesis was rejected at the $\alpha = 0.05$ level ($\log_e L_0 = -16925.01$, $\log_e L_1 = -16811.27$, $-2 \log_e \Lambda = 227.48$, $P < 0.0001$).

To better accommodate unequal substitution rates among lineages, a semi-parametric penalised likelihood (PL) rate smoothing with a truncated Newton algorithm was implemented to estimate relative ages of nodes using r8s (Sanderson, 2003). Penalised likelihood imposes constraints on rate variation combining a model allowing substitution rate variation among branches with a roughness penalty that mediates rate changes from branch to branch. A "smoothing parameter" that determines the relative contribution of each was determined by a cross-validation procedure. To generate a PL chronogram, we used MrBayes to generate a consensus phylogeny using the same priors as described above. Modeltest 3.0 (Posada & Crandall, 1998) was used to determine the appropriate nucleotide substitution model (GTR + I + G) and gamma shape required for r8s analysis. The outgroup *Rhopalthripoides* was pruned prior to r8s analyses. The 95% central distribution intervals for node-age estimates were generated by filtering post-burnin trees, retaining only those that had an identical topology to the consensus tree produced by our Bayesian analysis. This resulted in 258 sampled trees from the MCMC run. We used r8s to generate chronograms for each of these filtered trees. The resulting branch length estimates were used to calculate the ages of key nodes for each tree and the 2.5% right and left tails were then removed, leading to 95% central distribution limits which could be used as a measure of confidence for the point estimates (Schwarz *et al*., 2006).

Clock calibration

In the absence of even a modest amount of evidence bearing directly on gall-thrips divergence times, fossil *Acacia* pollen and biogeographical evidence was used to calibrate the phylogeny. Therefore, chronological conclusions are only

valid under the assumption that gall-thrips radiations must have coincided with host radiations. This is a reasonable proposal given that gall-thrips specialising on *Plurinerves* display a less-than-strict form of cospeciation between insect and host (Chapter II) and are therefore tightly affiliated with this resource. In addition, we aim to show that the 'ratio' of the ages of two calibration points in the gall-thrips phylogeny is similar regardless of the absolute timing of these events.

Divergence events were calibrated using combinations of fixed and/or constrained maximum and minimum times at the root node and an internal node based on fossil *Acacia* pollen records and biogeographical evidence. Each of the calibration points used was individually assessed in separate analyses to compare the appropriateness of the other calibration point, and thereby determining the ratio (or fit) of calibration points. That is, the root node or an internal node was calibrated independently as a fixed age or constrained between two ages. For these tests, the root node was fixed at 25 million years ago (mya) and 15 mya and the internal node at 5 mya. Additionally, the root was constrained between 10-15 million years (myr) or the internal node between 2-5 myr. Constrained calibration points were presumed to be a more realistic prior because the biogeographic and fossil evidence are given in approximate terms.

Calibration points were nominated in accordance with the following evidence. The appearance of fossil *Acacia* pollen the fossil record in Australia 25 mya (Martin, 1994) and the distribution of the *Acacia* subgenera *Phyllodineae* hosts to which gall thrips are confined, indicate that radiations of this group occurred in Australia no more than 15 mya (Truswell & Harris, 1982; Maslin *et al*., 2003). Therefore, the appearance of gall-thrips hosts presumably coincided with the appearance of ancestor of these arid-adapted *Acacia* during the Miocene; therefore we constrained the root node between 10-15 mya. A rapid increase in fossil *Acacia* pollen co-occurs with a period of pronounced arid-zone expansion in Australia over the last 3 myr (Martin, 1994; Crisp *et al*., 2004). Evidence indicates speciation in host *Acacia* section *Plurinerves* occurred over a relatively short period as widespread aridity developed in the early Quaternary over the last 2-5 myr (Maslin & Hopper,

1982; Clapperton, 1990; Byrne *et al*., 2002; Andrew *et al*., 2003; Miller *et al*., 2003). The common ancestor of the *K. rugosus* species complex specialising on extant *Plurinerves* species was therefore constrained to between 2-5 mya. The *K. waterhousei* species complex specialises on the same group of host species as *K. rugosus* and was therefore expected to have radiated contemporaneously.

To provide a convenient visualisation of historical host utilisation by gall-thrips, as gall-thrips are highly host specific (Crespi *et al*., 2004), and to indicate the relative age of the transition from *Juliflorae* to the novel ancestor of host section *Plurinerves*, ancestral states were also inferred using a stochastic character mapping approach (SIMMAP: Bollback, 2004). SIMMAP is a post-tree analysis program for making inferences about character evolution and implements a Bayesian method for mapping characters using stochastic substitution models (Nielsen, 2002). To avoid potentially spurious inferences based on a single topology, character histories were sampled from the posterior distribution of 1000 trees obtained from our MrBayes analyses. We used a data matrix of host utilization to calculate the probability of a shift to a *Plurinerves* ancestor. Gall-thrips host species assignments for the data matrix were taken from Crespi *et al*. (2004). *Plurinerves*, *Juliflorae*, or *Phyllodinae* host affiliation was coded from 0 to 2 respectively.

To provide an indication of the distribution of host affiliation with both *Juliflorae* and *Plurinerves* sections we substituted gall-thrips species with a very coarse categorisation of their sampling location and mapped them on to an area-cladogram. The sampling localities invariably represent one of many sites throughout the region they are distributed. However, many of the host distributions render the broad categorisation an appropriate indication of range.

Diversification rate

To investigate diversification rate changes we used a whole-tree asymmetry approach implemented in SYMMETREE (Chan & Moore, 2005). The approach is appropriate for use with trees including taxa below species-level and it is robust to polytomies. Divergences were expected to be marginal in some instances as mtDNA COI uncorrected “p” distances (see Appendix IV)

were below that between other gall-thrips species (Crespi *et al*., 2004; McLeish *et al*., 2007 in press). One lineage within the *K. rugosus* complex is now described as a separate species, *K. nicolsoni* (McLeish *et al*., 2006). Detection of among-lineage diversification rate variation compares observed differences in sister-group diversity to a null distribution of sister-group diversity. Diversification rate variation within the phylogeny is reported by a range of test statistics that vary in their sensitivity to nodal depth scales (Chan & Moore, 2005).

Diversification rate change along a branch can also be inferred. Two different diversification rate change *P*-statistics are calculated. Each calculation considers the detection of a shift along an internal branch of a three-taxon tree in different ways (Moore *et al*., 2004). The first, $P\Delta_1$, accounts for the differences between rate models and the second, $P\Delta_2$, for the possibility that the ingroup diversity might not be subject to a rate shift. The 'batch processing' option in SYMMETREE was applied using posterior probability distribution of 1000 Bayesian trees thinned from 10000. We used a random-resolution algorithm taxon-size sensitive (TTS) ERM branching model. The number of random resolutions was set to 100000 whole trees. To test the reliability of the single-tree analysis and the potential phylogenetic uncertainty associated with it, we batch processed a random sub-sample of 100 trees generated from posterior probability replicates of the Bayesian analysis.

To test the hypothesis of diversification rate change further, we also used a method for detecting multifurcations, or molecular polytomies, using likelihood ratio tests (Goldman & Whelan, 2000; Slowinski, 2001), expecting a reasonable coincidence of branch lengths not significantly different from zero with regions of diversification rate change. Low-level node support of internal branches in phylogenies can be a result of insufficient data or an artifact of short periods of rapid speciation, during which little or no informative signal is trapped. We suspected a series of poorly supported branches corresponded to rapid speciation as inclusion of additional markers did not improve node support at these nodes in several instances. We used Modeltest (Posada & Crandall, 1998) to estimate the substitution rate characteristics of each partition used in our phylogenetic analysis and used likelihood ratio tests implemented

in PAUP* to detect branch lengths not significantly different from zero for separate and all partitions combined.

A lineage by time diversification plot was used to show differential rates of gall-thrips lineage diversification that specialise on either *Plurinerves* or *Juliflorae*. Lineage frequencies per time were counted using the penalised likelihood chronogram generated by Sanderson's (2003) r8s program. The plot includes taxa most likely below that of species status where a large majority of known yet undescribed gall morpho-types (McLeish *et al*., 2006) have been included. We presume that several known morpho-types specialise on *Juliflorae* host species, that were not sampled, are only marginally underrepresented.

Phylogeographical analyses

Templeton's (1998) nested clade approach was used to investigate whether historical processes in *K. nicolsoni* are compatible with vicariance or dispersal events or both. *Kladothrips nicolsoni* was nominated as a suitable candidate for several reasons: *K. nicolsoni*, recently included in the *K. rugosus* complex, has been conferred species status (McLeish *et al*., 2006); the host on which *K. nicolsoni* specialises, *Acacia papyrocarpa,* is distributed broadly across the east-west axis of Australia, and therefore connects host ranges to the eastern and western margins of the continent; and *A. papyrocarpa* is subject to minimal range overlap with other *K. rugosus* complex host species, reducing the possibility of sampling cryptic sympatric populations (Crespi *et al*., 2004). The host range of *A. papyrocarpa* was also the most accessible making a thorough sampling regime practical.

This approach separates population structure from historical processes and allows the testing of hypotheses of geographical associations including population fragmentation, range expansion, restricted dispersal/gene flow, and long distance dispersal. AMOVA was used to test the significance of population substructure over the range of *K. nicolsoni* to determine levels of gene flow among populations of this species. To maximise DNA yield each tissue extraction comprised all individuals in a single gall, the brood of one female (Chapman *et al*., 2000). Voucher specimens of this taxon have been

deposited in the Australian National Insect Collection (ANIC) at CSIRO Entomology in Canberra. Extraction, PCR, and alignment protocols are given in McLeish *et al*. (2006).

Genealogical relationships of 48 COI haplotypes of *K. nicolsoni* were estimated. Maximum parsimony haplotype networks were generated using the TCS computer program (Clement *et al*., 2000). The haplotype network was used to develop a series of nested clades (Templeton, 1998). GeoDis version 2.2 (Posada *et al*., 2000) was used to detect non-random associations between geographic distribution and genetic variation via random, two-way contingency permutation analysis. The clade distance (D_c) measures the geographical range of a nominated clade. The nested clade distance (D_n) measures the average distance that a haplotype lies from the geographical centre of all haplotypes from the same clade. Differences in these measures between tips and the next immediate interior clade (I-T value) are important for discriminating hypotheses of geographical structuring of the genetic variation and determined by testing a null hypothesis of random geographical distribution for all clades within a nesting category. To test the significance ($\alpha = 0.05$ level) of D_c and D_n, resampling replicates were permutated 10000 times. Criteria used to interpret the significance of the various nested relationships are given in the most recent inference key (http://darwin.uvigo.es/download/geodisKey_11Nov05.pdf), and were used to identify hypotheses of historical processes.

The significance of the COI gene fragment covariance among and within populations of *K. nicolsoni* was tested using AMOVA, non-parametric permutation procedures (Excoffier *et al*., 1992). Covariance components and associated fixation indices (F_{ST}) were obtained using 10000 permutations implemented in ARLEQUIN version 2.0 (Schneider *et al*., 2000). We accommodated unequal mutation rates among sites (Chapter II) in the COI data by setting a gamma shape parameter, estimated in Modeltest, to be 0.8. The approach requires *a priori* groupings of the haplotype samples (Table 1). We grouped the data into a series of different analyses varying in levels of 'geographic coarseness' of populations. For instance, we varied our analyses by nominating only 2 hypothetical populations of haplotypes distributed over

the entire species range, up to 9 populations that largely corresponded to the sampling locations (Table 1). Samples from Buckleboo, Kolendo, and Yardea were collapsed into one population due to their proximity to one another. The sample from Iron Knob was included with the Middleback samples. Phylogenies inferred using maximum parsimony and Bayesian inferences for the 48 mitochondrial COI sequences employed the search parameters described above. The next most ancestral lineage to *K. nicolsoni*, *K. rugosus* on *A. papyrocarpa*, was used as an outgroup.

RESULTS

Phylogenetic analysis

The maximum parsimony and Bayesian phylogenetic analyses are largely congruent with one another and show a high level of support for each of the clades containing the *K. rugosus* and *K. waterhousei* species complexes (Figures 1 & 2). The clade comprising *Kladothrips acaciae* (Moulton), *Kladothrips ellobus* (Mound), *Kladothrips maslini* (Mound, Crespi, & Kranz) and the *K. rugosus* complex, closely parallels host usage by the sister-clade comprising *Kladothrips harpophyllae* (Mound, Crespi, & Kranz), *Kladothrips hamiltoni* (Mound & Crespi), *Kladothrips rodwayi* (Hardy) and the *K. waterhousei* complex. This is consistent with previous phylogenies (Morris *et al*., 2001) where a taxon from each clade specialises on the same host species.

Monophyly of the *K. waterhousei* and *K. rugosus* complexes was apparently violated by the presence of *K. rodwayi* and *K. maslini* respectively. We suspect long-branch attraction, due to incomplete sequence data for the *K. waterhousei* complex at EF-1a and *wingless* gene regions (Wiens, 2005), has resulted in this positioning of *K. rodwayi* (Figures 1 & 2). To test this assumption, we partitioned the sequence data to include only those gene regions with a full complement of loci across all taxa and inferred a phylogeny by implementing maximum parsimony. This inference indicated that *K. rodwayi* was a sister-taxon to the *K. waterhousei* complex. We also generated a Bayesian consensus with putative host races of *K. waterhousei* and *K. rugosus*

(Chapter II) with the resulting tree indicating *K. rodwayi* to be a sister-taxon to the *K. waterhousei* complex. Monophyly of the *K. rugosus* complex becomes invalid by the presence of *K. maslini*, though support values at bifurcations in this vicinity were weak. Additionally, the recent elevation of a *K. rugosus* host race inhabiting *A. papyrocarpa* to species status, *K. nicolsoni* (McLeish *et al*., 2006), also invalidates monophyly of this species complex but is consistent with the clade comprising multiple putative species.

Chronology

Penalised likelihood analysis using our Bayesian inference revealed contemporaneous radiations of two gall-thrips clades, one comprising the *K. rugosus* complex and the other *K. waterhousei*, *Kladothrips intermedius* (Bagnall), and *Kladothrips habrus* (Mound) complexes. We explored outcomes of r8s tests by assigning different combinations of fixed and constrained ages to the root and an internal node (Table 2) to assess the ratio (or fit) between each of our calibration priors. When the root node was fixed at 25 mya, approximating the appearance of fossil *Acacia* pollen in Australia, the internal node corresponding to the presumed period of rapid speciation of *Plurinerves*, was dated at nearly 9 mya. This date approaches the period when arid-adapted *Acacia* are believed to have originated and therefore 25 mya was regarded as an upper limit for the age of the root node.

Fixing the age of the root node at 15 mya, and then constraining the age between 10-15 mya in three separate tests resulted in the age of the appearance of the common ancestor of the *K. rugosus* and *K. waterhousei* complexes being dated at between 3.3 mya and 5.3 mya (Table 2). Furthermore, we enforced a fixed divergence time of 5 mya at this node in one analysis and constrained this point between 2 mya and 5 mya in another test. The root node date estimates were 9.3 mya and 13.7 mya respectively (Table 2). Therefore, the ratio of the age of the root node and the common ancestor of the *K. rugosus* and *K. waterhousei* complexes were consistent with one another between the independent analyses.

To generate our chronogram (Figure 3), we used a gamma shape distribution value of 0.5316 generated using Modeltest 3.0 (Posada & Crandall,

1998). The age at the root and at the common ancestor of the *K. rugosus* complex was constrained between 10 and 15 mya and 2 and 5 mya respectively. The cross validation analysis yielded a smoothing value of 3.981072. The chronogram indicated that the common ancestors of the *K. rugosus* and *K. waterhousei* complexes arose at approximately the same time between 3.38 and 6.05 mya. The age of the root of the phylogeny was dated at between 10.00 and 10.43 mya. Host affiliation inferred using a stochastic character mapping approach implemented in SIMMAP indicated a host switch to a *Plurinerves* ancestor (the probability of a *Plurinerves* ancestor at this node = 99.9%) at approximately 7.5 mya.

The area-cladogram of species sampling locations indicated a preponderance of eastern-distributed species specialising on *Plurinerves* hosts. Lineages affiliated with *Juliflorae* tend to be located in central-western regions of Australia (Figure 4).

Diversification rate

We used likelihood ratio tests to detect branch lengths that were not significantly different than zero to assess the possibility of hard molecular polytomies. The genetic data were partitioned into six to generate Bayesian inferences. As different partitions showed branch length variation, we mapped branch lengths not significantly different from zero (at the $\alpha = 0.05$ level) for 6/6 partitions, 5/6 partitions, 4/6 partitions, and no partitions on to our Bayesian consensus phylogram (Figure 4). There was a high density of internal and terminal branch lengths not significantly different from zero for 6/6, 5/6, and 4/6 partitions within the *K. rugosus* and *K. waterhousei* complexes. For the 5/6 partitions of branch lengths not significantly different from zero, the non-zero length partition was always derived from the mitochondrial DNA data (COI & 16S), which might be expected given that these gene regions have higher substitution rates than the other partitions. When the data were pooled together (i.e. no partitions), branch lengths not significantly different from zero always coincided with all or a high proportion of branch lengths not significantly different from zero indicated by the partitioned analyses.

SYMMETREE performs seven slightly different alternatives of diversification rate tests, each progressively more sensitive to different nodal depth scales (Chan & Moore, 2005). When testing for rate variation within a tree, significant tree asymmetry (Figure 4) was detected through the most to least sensitive test-statistics (single-tree $0.0001 < P < 0.0315$; batch processing $0.000 < P < 0.000$). Whole-tree analysis for the detection of tree asymmetry indicated that lineages within the gall-thrips phylogeny diversified at significantly different rates. Diversification rate shifts inferred from the best Bayesian consensus phylogeny are conditional on its accuracy and also implicitly assume full representation and/or random sampling of gall-thrips taxa. The phylogeny was lacking some gall-thrips taxa but was assumed not to compromise the analysis as sampling included a large majority of known types.

Given significant levels of tree asymmetry, branches along which putative rate shifts occurred can be identified. The significant rate shift identified in a branch in the single-tree analysis coincides with the node of the most recent ancestor of the *Kladothrips antennatus* (Moulton) lineage ($P\Delta_1 = 0.0363636$ and $P\Delta_2 = 0.0476392$). Substantial but not significant rate shifts were identified at the most recent ancestral branch to the *Kladothrips morrisi* (Mound, Crespi, & Kranz) lineage ($P\Delta_1 = 0.0583$ and $P\Delta_2 = 0.075$) and most recent ancestral branch to the *K. rodwayi* lineage ($P\Delta_1 = 0.066$ and $P\Delta_2 = 0.066$). Of the 100 replicates in our batch processing analysis used to account for phylogenetic uncertainty, the significant diversification rate shift, at the ancestor of *K. antennatus*, was consistently identified along the same branch although slight tree-topology changes occur among the replicate sample.

The lineage by time diversification plot (Figure 5) shows differential rates between gall-thrips that specialise on *Plurinerves* and *Juliflorae*. Known taxa that inhabit *Juliflorae* hosts not represented in the phylogeny comprise a small proportion of the total known types (approximately 5% of known types. See Crespi *et al*., 2004), and therefore the general trend in diversification rates would not alter substantially. The plot indicates lineages that specialise on *Plurinerves* host species diversify at a higher rate than those on *Juliflorae* hosts and this is contemporaneous with a period of exaggerated aridification during the Quaternary over the last 4 myr.

Nested clade and AMOVA analysis

From the 48 individual *K. nicolsoni* isolates, with 618 base pairs for each, sampled across 13 sites throughout the range of the host *A. papyrocarpa* (Table 1), we obtained 24 haplotypes. Each sampling locality did not contain unique haplotypes (Figure 6). The nested haplotype network was generated using a connection limit of 95% and is shown in Figure 7. In the total cladogram, random, two-way contingency permutation analysis indicated a significant association ($P < 0.05$) between haplotype and geographic location in clades 2-1 and 1-1 (Table 3).

Significantly small D_c and (I-T) D_n and significantly large D_n values in clade 2-1 were detected as restricted gene flow/dispersal but with some long distance dispersal over intermediate areas not occupied by the species; or past gene flow followed by extinction of intermediate populations. However, a less ambiguous significant association was detected in clade 1-1. Significantly large interior vs. tip (I-T) D_n and D_n values and a significantly small D_c value indicated past gradual range expansion followed by fragmentation.

The 48 isolates from 13 sites containing 24 haplotypes were grouped into populations at various 'proximity' scales. A total of 9 geographical populations were nominated for the AMOVA test as some populations were collapsed due to very close proximity to another site. The F_{ST} and covariance components altered only marginally in the other series of tests using various levels of 'population coarseness.' The results (Table 4) indicated a substantial percentage of the variation occurred within populations (85.8%) rather than among them (14.2%). The fixation index among the nine populations was $F_{ST} = 0.142$. The covariance components and F_{ST} were tested by permutating haplotypes among populations. The P value ($P = 0.007$) is taken as the proportion of two randomly chosen haplotypes from a population having a probability less then or equal to choosing from the group.

Maximum parsimony and Bayesian phylogenies were inferred using a COI gene fragment from each of 48 isolates and these genealogies were polytomy rich (and therefore not given here) except that for a bifurcation between the Western Australian and the eastern distributed populations of *K. nicolsoni*. Here, the ancestral outgroup, *K. rugosus* inhabiting *A. papyrocarpa*

(McLeish *et al*., 2006), grouped closest to the Western Australia distributed population of *K. niclosoni*.

DISCUSSION

Under the assumption of a thrips/*Acacia* co-radiation, chronological conclusions indicate the common ancestor of gall-thrips arose after the commencement of long-term desertification in Australia. The colonisation of a novel ancestral host related to the extant *Plurinerves* occurred before the inferred onset of more rapid desert expansion and climatic cycling over the Pleistocene. This host shift appears to have afforded greater opportunity for gall-thrips diversification on *Plurinerves* compared to species specialising on an alternative host section. Sequence divergences not consistent with more dramatic morphological variation (e.g. gall structure) amongst gall-thrips specialising on *Plurinerves* suggest this group has been subject to a relatively recent and rapid speciation episode. Historical population-level events indicated patterns of host plant vicariance, cladogenesis, or both and this is consistent with processes of climatic cycling during the Quaternary.

Phylogenetics

Maximum parsimony and Bayesian inferences indicated two well-supported, well-resolved monophyletic clades one comprising *K. ellobus*, *K. acaciae*, and the *K. rugosus* complex, and the other comprising *K. hamiltoni*, *K. harpophyllae*, and the *K. waterhousei*, *K. habrus* and *K. intermedius* complexes (Figures 1 & 2). These taxa are all affiliated with *Plurinerves* hosts. However, each of the clades includes either *K. maslini* or K. *rodwayi*, lineages that have undergone a host-switching event (Crespi *et al*., 1998) (Figure 3). Evidence of a less than strict form of cospeciation between these gall-thrips clades and the host species they share (Chapter II) implies that underlying processes impacting diversification might act in tandem for both clades on highly-related *Plurinerves*. This is in contrast to lineages inhabiting more distantly related host species.

Indications of processes driving gall-thrips diversification acting on the *K. rugosus* and *K. waterhousei* complexes might be expressed differently in *K. rodwayi* and *K. maslini*. These two species inhabit phylogenetically distant host taxa (Pedley, 1987; Maslin, 2001), are mesic-zone distributed (Morris *et al*., 2001; Kranz *et al*., 2002), and share secondarily acquired plesiotypic life history strategies. The inferred host switches of the *K. rodwayi* and *K. maslini* lineages might represent a shift to enemy free space (Ehrlich & Raven, 1964; Crespi *et al*., 2004). Phylogenetic inference suggests *Koptothrips* (Bagnall) species specialise on gall-thrips species (Crespi *et al*., 2004) and that as a consequence might also diversify in parallel with the host thrips after the host shift (Brown *et al*., 1995; Eubanks *et al*., 2003) and this is consistent with the presence of *Koptothrips* parasitising *K. rodwayi* galls. Very little life history information has been collected for *K. maslini* in contrast to that for *K. rodwayi* discussed below.

The inferred host shift of an ancestor of *K. rodwayi* apparently resulted in a loss of soldiers (sociality), the retention of small gall size, small brood sizes similar to social species, and adult eclosion in the gall before dispersal (Kranz *et al*., 2002). The phylogram (Figure 4) shows a relatively short terminal branch for the *K. rodwayi* lineage. However, the striking life history shift and presumed subsequent bottleneck effects resulting from the host switch would be expected to influence the level of sequence divergence observed in the *K. rodwayi* branch. This level of differentiation, assuming the sampled genetic change reflects observed phenotypic change (Figure 4) might help explain why *K. rodwayi* has retained some features of social species in this clade, including small brood numbers and small gall sizes.

Levels of sequence divergence among the complexes do not appear to be a good predictor of morphological (gall structure) or life history variation. Life history shifts appear to be more acute subsequent to a host-switching event. Dramatic life history shifts have also coincided with gall-thrips association with *Plurinerves* hosts. For example, gall-thrips on *Plurinerves* have evolved behaviourally and morphologically specialised defensive castes, cofounding between male and female, and adult eclosion outside of the natal gall (Crespi *et al*., 2004). The proliferation of gall-thrips lineages on

Plurinerves hosts contrasts with those on *Juliflorae* hosts and suggests that each group was subject to variation in processes affecting diversification that might be linked to host distributions/geography. Host-related races present in the species complexes inhabiting *Plurinerves* do not occur for the *K. maslini* and *K. rodwayi* lineages on more distantly related hosts distributed in non-arid climates. Host plant diversity has been found to play a key role underlying the rates at which host-switching, and therefore insect diversification, can take place (Craig *et al*., 1994; Mendonca, 2001). However, the opportunity for increased diversification after the successful colonisation of a novel niche need not necessarily follow and perhaps the processes driving diversification might differ between host groups. Taken together, these patterns suggest that diversification in gall-thrips is closely linked to host evolution in addition to host affiliation.

Chronology

We estimated absolute timing of lineage diversification and mapped host affiliation onto our Bayesian consensus tree (Figure 4). In the absence of direct evidence of gall-thrips divergence times, co-radiation between thrips and *Acacia* has been treated as an assumption and the ratio of timing between the origin and radiation of gall-thrips specialising on *Plurinerves* hosts tested. Independent analysis of each calibration point supported the timing of the alternative calibration date and a chronogram was generated using both constraints. Penalised likelihood estimates and ancestral host affiliation inferences show a host switch to a *Plurinerves* ancestor approximately 7.5 mya, before more extreme arid-zone expansion during the Quaternary. The common ancestors of the *K. rugosus* and *K. waterhousei* complexes were contemporaneous both occurring approximately 3.6 mya. The coincidental diversification of the two complexes, each specialising on the same set of *Plurinerves* host species, suggest that a component of diversification opportunities for gall-thrips was a consequence of host range expansion and host speciation.

Diversification rate changes

Our evidence supports Hopper and Gioia's (2004) prediction that phylogenetic pattern showing unresolved relationships towards the terminal branches of a tree attests to rapid increase in diversification. The high incidence of molecular polytomies and short branch lengths towards the terminal ends of the phylogeny generally occur for gall-thrips lineages that specialise on *Plurinerves* and not *Juliflorae*. The presence of hard molecular polytomies (Figure 3) indicated that poor resolution within the *K. rugosus* and *K. waterhousei* complexes (Figures 1 & 2) might be the outcome of rapid speciation in these lineages (Goldman & Whelan, 2000; Slowinski, 2001).

Furthermore, diversification rate variation analyses identified significant tree asymmetry at a branch believed to represent the transition from gall-thrips lineages that display a plesiotypic life history (Figure 3). The four species, *Kladothrips zygus* (Mound, Crespi, & Kranz), *Kladothrips pilbara* (Mound, Crespi, & Kranz), *Kladothrips schwarzi* (Mound, Crespi, & Kranz), and *K. antennatus* are considered to be similar in biology to ancestral gall-inducing thrips. These species display the most 'primitive' morphology and gall structure, inhabit *Juliflorae* hosts, and are distributed in the most arid regions of northwestern and central Australia (Crespi *et al*., 2004). *Juliflorae* such as *Acacia aneura* (F. Muell ex. Benth), supports *K. antennatus* and three other centrally distributed species, *Kladothrips arotrum* (Mound), *Kladothrips sterni* (Mound, Crespi, & Kranz), and *Kladothrips tepperi* (Uzel).

The underlying causes of the diversification rate change before the origins of the *K. rugosus* and *K. waterhousei* complexes are difficult to test. The inferred rate change occurred (Figure 4) early in the evolution of gall-thrips *Acacia*, and therefore might represent a footprint of central arid-zone expansion before the proposed *Plurinerves* host range expansion on the arid-zone periphery resulting from climatic cycling. The central Australian desert is known to have originated as severe aridity developed during the Pliocene from approximately 7 mya (Maslin & Hopper, 1982; Wasson, 1982; Clapperton, 1990; Markgraf *et al*., 1995). Truly arid-zone host populations might be less evolutionarily labile than populations in semi-arid regions. Phenotypic diversity (Crespi *et al*., 2004) and species richness appear to have been

amplified on the *Plurinerves* lineage compared to lineages supported by centrally distributed *Juliflorae* (Figure 5) over the last 4 myr assuming our chronogram is a reasonable approximation. It is possible that fragmentation (see discussion below) featured more prominently in the *Plurinerves* during the most recent and extensive speciation event. The *Juliflorae* might have been able to maintain gene flow sufficient to arrest fragmentation effects apparently experienced by the *Plurinerves*.

Phylogeography

Historical patterns of population biogeography for *K. nicolsoni* inferred from nested clade analysis indicated: (i) past gradual range expansion followed by fragmentation; and/or (ii) restricted gene flow/dispersal but with some long distance dispersal over intermediate areas not occupied by the species or past gene flow followed by extinction of intermediate populations (Table 3). Long distance dispersal by thrips is possible (McLeish *et al*., 2003) so the hypothesis of restricted gene flow cannot be discounted. These significant results are primarily the outcome of haplotype distributions to the west of the Nullabor Plain and populations to the east of the Plain (Figure 6). Floral communities on desert margins in Australia would have been sensitive to glacial extremes and climatic cycling (Hopper, 1979; Crisp *et al*., 2001; Burgman, 2002; Chapple & Keogh, 2004) and speciation in the sclerophyllous taxa was partly due to population fragmentation and lability induced by these upheavals (Truswell & Harris, 1982; Anderson, 1994; Markgraf *et al*., 1995). Restricted gene flow and consequent differentiation among gall-thrips populations would be promoted by fragmented host distributions.

The current range of *K. nicolsoni* on *A. papyrocarpa* is patchy and only extends to well before the western extreme of the Nullabor Plain and beyond its eastern boundary well into northeastern South Australia (Figure 6). The fragmentation of ancestral gall-thrips populations suggests speciation in gall-thrips is partly a consequence of vicariant processes. The fixation index (F_{ST} = 0.142) and genetic variance components (Figure 4) indicated restricted gene flow or recent shared ancestry. Negligible gene flow among *K. nicolsoni* populations is consistent with historical hypotheses that imply allopatry.

Furthermore, different gall-thrips species that persist in sympatry on the same host species, such as on *A. papyrocarpa*, might be explained by diversifying on the same host species in allopatry proceeded by secondary contact.

Lack of informative sites in the haplotype sample prohibited reliable coalescence analyses. Therefore, we have no means of temporally polarizing the haplotype cladogram. The haplotype network (Figure 7) shows a frequent, widespread haplotype that was sampled from the eastern extent of the range and not in western Australia (Figure 6), indicative of the geographical origin (Templeton, 1998). This might imply that range expansion occurred towards the west and that this might reflect host expansion inertia. The area-cladogram (Figure 4) indicates lineages that specialise on *Plurinerves* tend to the east of the continent while those species on *Juliflorae* are located to the west and centrally. The host range map of *Plurinerves* hosts (Figure 6) shows a high concentration of species to the east and southwest of the continent but none in central Australia. Gall-thrips species richness might not necessarily overlap host richness as phytogeography of *Plurinerves* and *Juliflorae* (Hnatiuk & Maslin, 1988) shows relatively high richness in the central region of Australia. The distribution history of an organism is expected to be an outcome of dispersal and vagility (Trewick, 2000; Bouchard & Brooks, 2004; Cook & Crisp, 2005). As the dispersal ability of gall-thrips is presumed weak, these asymmetries in gall-thrips distributions suggest a directional component either to dispersal, host expansion, or both.

Conclusions

This work has addressed hypotheses connecting gall-thrips phylogeny with host plant evolution and suggests that host expansion, host geography, and climate change have important consequences for gall-thrips diversification. A pronounced speciation episode for gall-thrips lineages affiliated with *Plurinerves* hosts appears to have commenced between 3 and 6 mya. The inferred host-plant switch to a *Plurinerves* ancestor relatively early in the evolution of gall-thrips, preceded the onset of pronounced diversification for this lineage. In addition to cospeciation and host switching evident for this group, *Acacia* cladogenesis would provide an expanding resource and

unoccupied ecological niches to exploit. These patterns agree with the climatic cycling during the Quaternary contriving an opportunity for some phytophagous insects, associated with a particular flora, to diversify at heightened rates with respect to others. This work suggests a component of the plant-feeding insect diversity in Australia has been passively driven by host evolution.

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Table 1: Description and locations of collection sites for *K. nicolsoni* specialising on *A. papyrocarpa* used in nested clade and AMOVA analyses. The COI mtDNA fragment haplotype samples are from 9 populations. Roman numerals indicate haplotype.

Sample	Haplotypes	Location	Pops.	Latitude	Longitude
1	XIX	Middleback	1	32 56 45 S	137 23 24 E
2	III	Middleback	1	32 56 45 S	137 23 24 E
3	XIX	Middleback	1	32 56 45 S	137 23 24 E
4	I	Middleback	1	32 56 45 S	137 23 24 E
5	VIII	Middleback	1	32 56 45 S	137 23 24 E
6	XIX	Middleback	1	32 56 45 S	137 23 24 E
7	XIX	Middleback	1	32 56 45 S	137 23 24 E
8	XIX	Middleback	1	32 56 45 S	137 23 24 E
9	VII	Middleback	1	32 56 45 S	137 23 24 E
10	XIX	Middleback	1	32 56 45 S	137 23 24 E
11	XV	Middleback	1	32 56 45 S	137 23 24 E
12	XXIV	LkGillies	2	33 01 35 S	136 48 23 E
13	XIX	LkGillies	2	33 01 35 S	136 48 23 E
14	X	LkGillies	2	33 01 35 S	136 48 23 E
15	XVI	Kookartha	3	31 17 27 S	135 16 41 E
16	XII	Kookartha	3	31 17 27 S	135 16 41 E
17	XX	Buckleboo	4	32 45 55 S	136 02 44 E
18	XIX	Kolendo	4	32 25 14 S	136 18 05 E
19	IX	IronKnob	1	32 48 55 S	137 11 06 E
20	XIX	Yardea	4	32 21 42 S	135 39 21 E
21	XVIII	Kingoonya	5	32 22 12 S	135 25 06 E
22	VI	Kingoonya	5	32 22 12 S	135 25 06 E
24	XXII	Hiltaba	6	32 15 10 S	135 15 27 E
25	XIX	Hiltaba	6	32 15 10 S	135 15 27 E
26	V	Hiltaba	6	32 15 10 S	135 15 27 E
27	XI	Hiltaba	6	32 15 10 S	135 15 27 E
28	XIX	Wirrulla	7	32 18 01 S	135 00 54 E
29	XIV	Wirrulla	7	32 18 01 S	135 00 54 E
30	XVII	Caiguna	8	32 21 47 S	124 43 46 E
31	XVII	Caiguna	8	32 21 47 S	124 43 46 E
32	XVII	Caiguna	8	32 21 47 S	124 43 46 E
33	VI	Caiguna	8	32 21 47 S	124 43 46 E
34	VI	Caiguna	8	32 21 47 S	124 43 46 E
35	XXI	Caiguna	8	32 21 47 S	124 43 46 E
36	XXIII	Caiguna	8	32 21 47 S	124 43 46 E
37	XXIII	Caiguna	8	32 21 47 S	124 43 46 E
38	XXIII	Caiguna	8	32 21 47 S	124 43 46 E
39	IV	CopperHills	9	27 57 27 S	134 14 04 E
40	VI	CopperHills	9	27 57 27 S	134 14 04 E
41	XIX	CopperHills	9	27 57 27 S	134 14 04 E
42	XIII	CopperHills	9	27 57 27 S	134 14 04 E
43	XIX	CopperHills	9	27 57 27 S	134 14 04 E
44	XIX	CopperHills	9	27 57 27 S	134 14 04 E
45	VI	CopperHills	9	27 57 27 S	134 14 04 E
46	VI	CopperHills	9	27 57 27 S	134 14 04 E
47	II	CopperHills	9	27 57 27 S	134 14 04 E
48	VI	CopperHills	9	27 57 27 S	134 14 04 E
49	XIX	CopperHills	9	27 57 02 S	134 19 17 E

Table 2: Penalised likelihood estimates of root node and internal node ages using our Bayesian consensus phylogram. The internal node corresponds to the most recent common ancestor of the *K. rugosus* species complex. Ages are in units of millions of years (myr). Rate smoothing values were calculated using a cross validation method implemented in Sanderson's (2003) r8s program.

Calibration Points (myr)	Smoothing value	Root age	Internal age
root: 25	14.454	25.00	8.97
root: 15	15.849	15.00	5.31
root: 10-15	13.183	10.01	3.61
internal: 5	69.183	13.66	5.00
internal: 2-5	6.918	9.31	3.38

Table 3: The results of the nested clade analysis of the *K. nicolsoni* COI mtDNA haplotypes. The nested clade haplotype network is given in Figure 7. The clade distance D_c measures the geographical range of the designated clade. The nested clade distance D_n measures the average distance that the haplotype lies from the geographical centre of all haplotypes of the same clade. The average distance between tip vs. interior clades for both distance measures is given and designated I-T. The connection limit was set to 95%. The superscripts 'S' and 'L' indicated the distance measure was significantly (α = 0.05) small or large respectively. Resampling replicates were permutated 10000 times. The number sequences follow the steps through the inference key and the hypothesis indicated: REF = past gradual range expansion followed by fragmentation; and LDD = restricted gene flow/dispersal with some long distance dispersal or GFE = past gene flow followed by extinction of intermediate populations. The grey shaded areas indicate interior clades.

Haplotypes			1-Step Clade			2-Step Clade			3-Step Clade		
No.	**D_c**	**D_n**	**No.**	**D_c**	**D_n**	**No.**	**D_c**	**D_n**	**No.**	**D_c**	**D_n**
I	0	0	1-13	0	314.6						
II	0	0	1-12	0	316.5						
			I-T	0	1.9	2-4	315.6	359.6			
III	0	414.6									
IV	0	350.5									
V	0	205.7									
VI	414.1	395.2	1-9	362.4	348.3						
I-T	414.2	71.5									
VII	0	0	1-11	0	369.9						
VIII	0	0	1-10	0	369.9						
			I-T	362.4	-21.6	2-3	350.6	346.6			
IX	0	211.9				I-T	35.0	-13.0	3-1	225.8	234.1
X	0	200.4									
XI	0	60.9									
XII	0	47.0									
XIII	0	430.4									
XIV	0	72.7									
XV	0	235.9									
XVI	0	47.0									
XVII	**0S**	**1002.0L**									
XVIII	0	73.7									
XIX	214.5	202.2									
I-T	169.3	**153.7L**									
1-2-3-5-15-NO-21-NO REF			**1-1**	229.5	240.0						
XX	0	0	1-6	0	155.9						
XXI	0	0	1-2	0	937.9						
XXII	0	0	1-3	0	62.9						
XXIII	0	0	**1-5**	**0S**	**937.9L**						
XXIV	0	0	1-8	0	0						
			I-T	229.5	**-421.8S**						
			1-2-3-5-6-7-8-YES LDD/GFE			2-1	229.5	226.3			
						2-2	0	209.8			
						I-T	229.5	16.4	3-2	347.8	318.4

Table 4: AMOVA design and results used to test the significance of population substructure over the range of the species to determine levels of gene flow among populations of *K. nicolsoni* inhabiting *A. papyrocarpa* are shown here. Covariance components and associated fixation indices (F_{ST}) were obtained using 10000 permutations among the 9 designated populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among Populations	8	10.657	0.12105	14.23
Within Populations	39	28.447	0.72914	85.77
Total	47	39.104	0.85046	

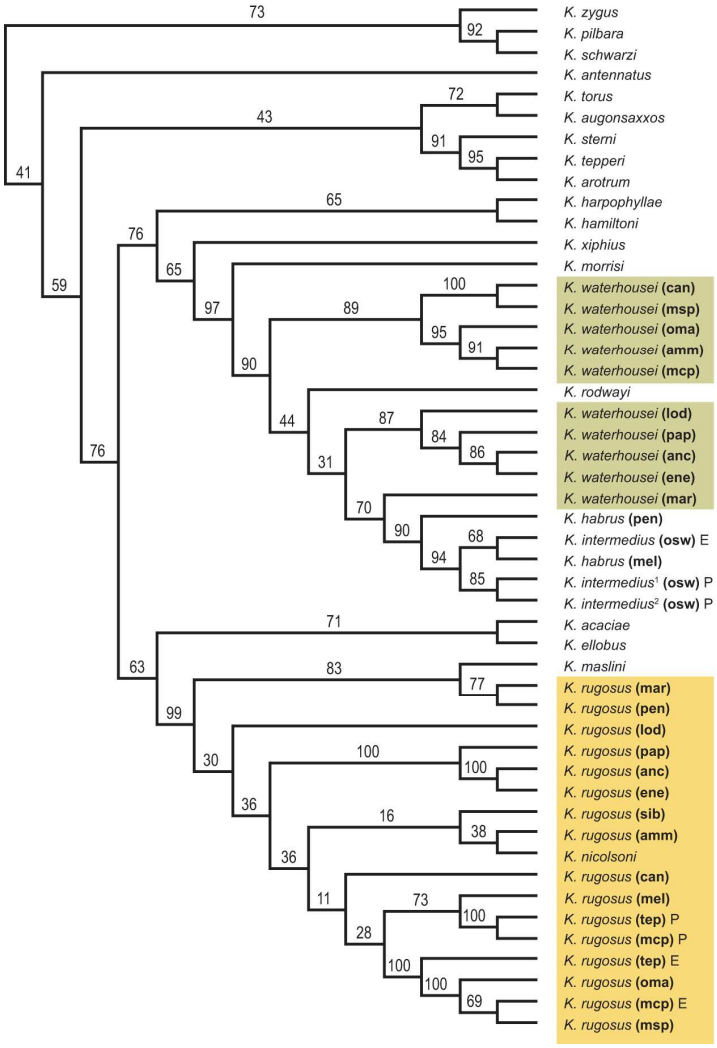


Figure 1: A bootstrap maximum parsimony phylogeny using COI, EF-1α, *wingless*, and 16S genes. A heuristic search with tree bisection-reconstruction (TBR) branchswapping, random addition of taxa 100 replicates per search and 10 trees held at each step), and 1000 bootstrap replicates was implemented in the construction of the tree. Abbreviations for *Acacia* host races are as follows: *A. ammophila* (amm), *A. ancistrophylla* (anc), *A. cana* (can), *A. enervia* (ene), *A. loderi* (lod), *A. maranoensis* (mar), *A. melvillei* (mel), *A. microcephala* (mcp), *A. microsperma* (msp), *A. omalophylla* (oma), *A. oswaldii* (osw), *A. papyrocarpa* (pap), *A. pendula* (pen), *A. sibilans* (sib), and *A. tephrina* (tep). Taxon codes are as follows: E = elongate and P = pouched gall-type.The coloured boxes indicate the *K. rugosus* and *K. waterhousei* species complexes.

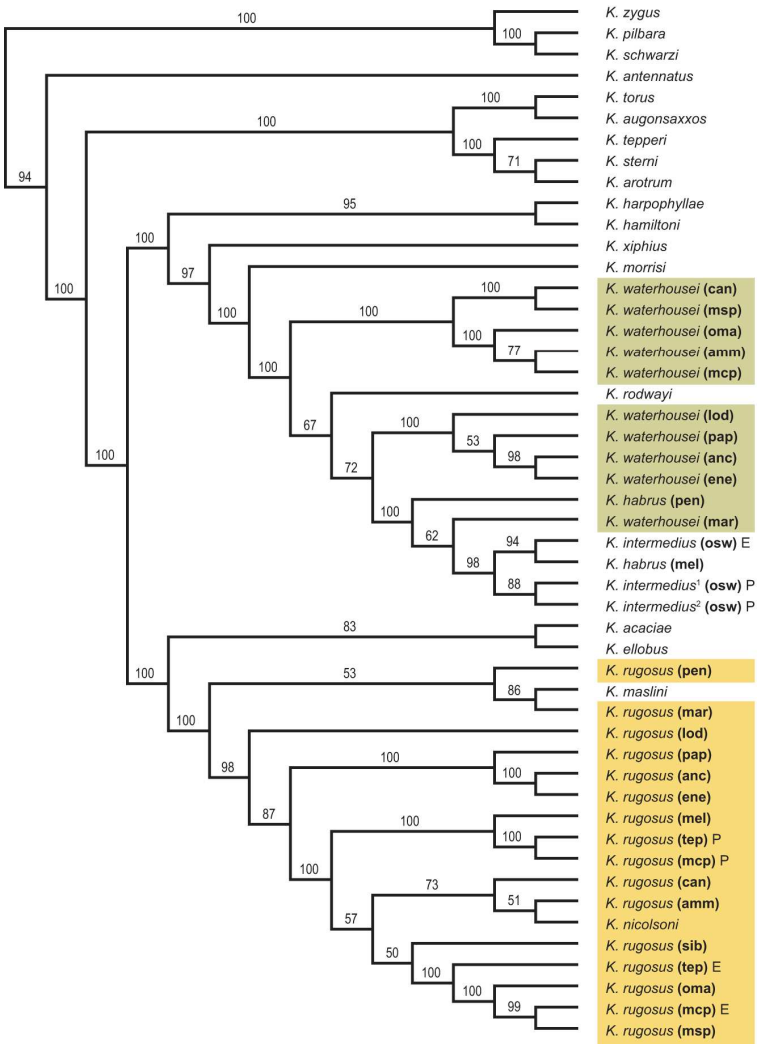


Figure 2: A Bayesian Consensus analysis of six separately modelled partitions comprising 1st, 2nd, and 3rd COI codons and separate EF-1α, *wingless*, and 16S sites. Posterior probabilities and mean branch lengths are derived from 3000 trees taken from a sample of 5 million generations, sampling every 500th generation. Abbreviations for *Acacia* host races are as follows: *A. ammophila* (amm), *A. ancistrophylla* (anc), *A. cana* (can), *A. enervia* (ene), *A. loderi* (lod), *A. maranoensis* (mar), *A. melvillei* (mel), *A. microcephala* (mcp), *A. microsperma* (msp), *A. omalophylla* (oma), *A. oswaldii* (osw), *A. papyrocarpa* (pap), *A. pendula* (pen), *A. sibilans* (sib), and *A. tephrina* (tep). Taxon codes are as follows: E = elongate and P = pouched gall-type. The coloured boxes indicate the *K. rugosus* and *K. waterhousei* species complexes.

Figure 3: A penalised likelihood chronogram of our Bayesian consensus tree. The dark tan coloured branch indicates an ancestral affiliation with those of host section *Phyllodinae*; tan coloured branches indicate *Juliflorae* ancestral host affiliation; and green coloured branches indicate *Plurinerves* ancestral host affiliation. Red node circles show constrained calibration points between 10-15 mya and 2-5 mya. A coarse-scale Australian location of each taxon is indicated as follows: W = west; C = central; S = southerly; and E = eastern. The coloured boxes indicate the *K. rugosus* and *K. waterhousei* species complexes. The time scale is in millions of years ago (mya).

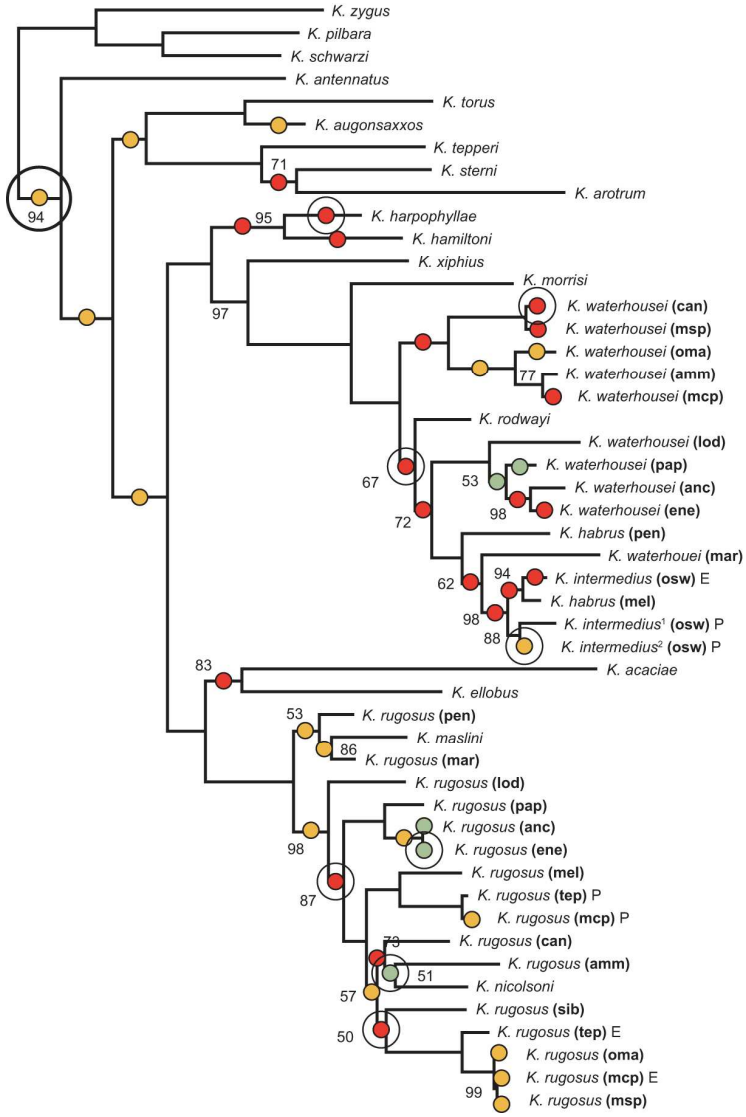


Figure 4: Bayesian Consensus phylogram using six separately modelled partitions comprising 1st, 2nd, and 3rd COI codons and separate EF-1α, *wingless*, and 16S gene regions showing significantly zero length branches derived from likelihood ratio tests of different gene partitions. Green dots indicate 6/6 partitions with significantly zero length branches, red dots indicate 5/6 partitions, and yellow dots indicate 4/5 partitions. The intermediate sized open circles indicate significant zero length branches when the data was not partitioned. The large open circle shows the branch where a significant diversification rate change was detected. See Figures 1 & 2 for taxa abbreviations.

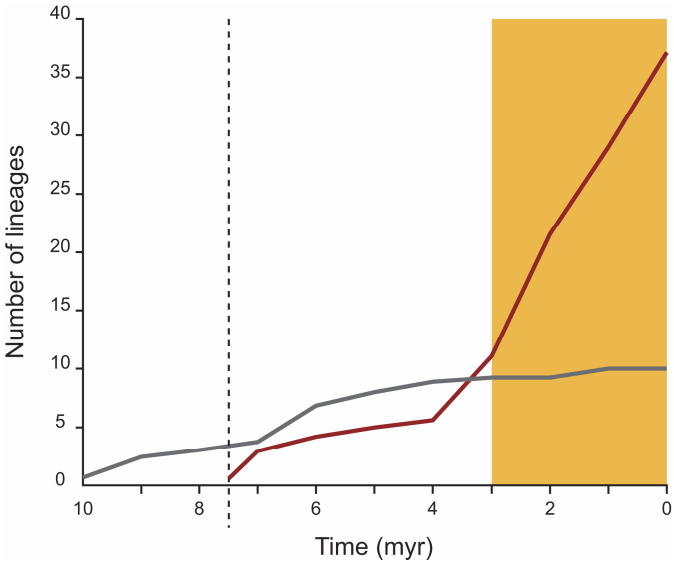


Figure 5: Lineage by time diversification plot showing differential rates of gall-thrips diversification that specialise on either *Plurinerves* (red line) or *Juliflorae* (grey line). The gold rectangle approximates the Quaternary period and the dotted line indicates the inferred host switch to a *Plurinerves* ancestor. Lineage frequencies were counted using the penalised likelihood chronogram (Figure 3).

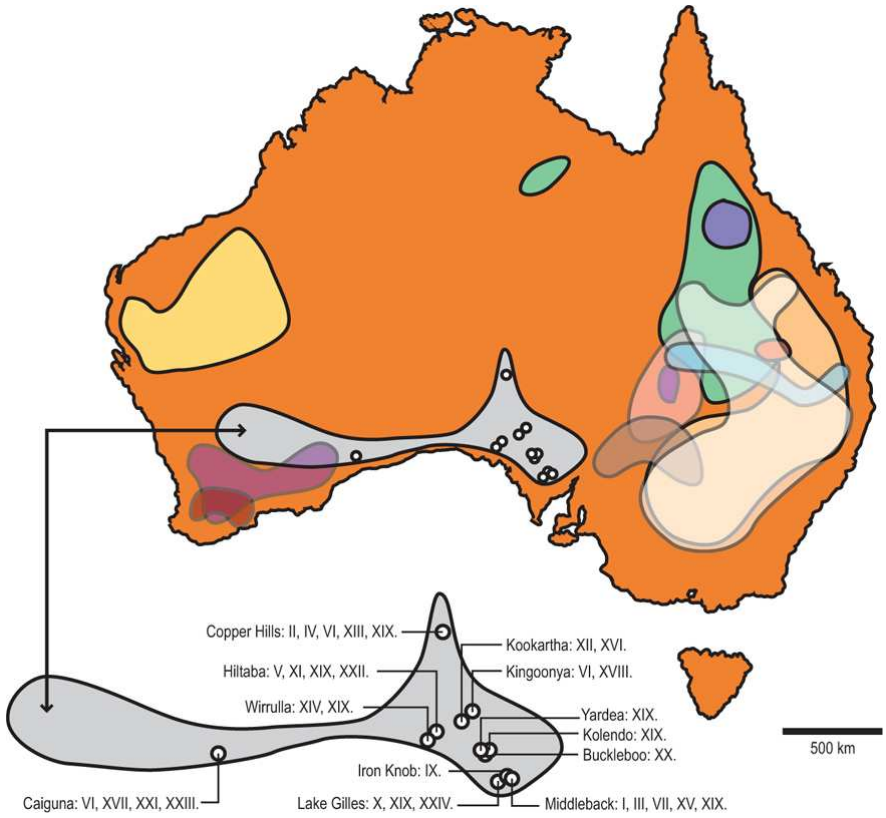


Figure 6: The species ranges (simplified from Maslin, 2001) of *Plurinerves* hosts of the *K. rugosus* and *K. waterhousei* complexes. Distribution ranges' of different *Acacia* species are show by various overlapping outlined areas. The highlighted host species range is that of *A. papyrocarpa*. White circles show *K. nicolsoni* (formerly *K. rugosus*) sampling sites with the haplotypes collected from each of them labelled.

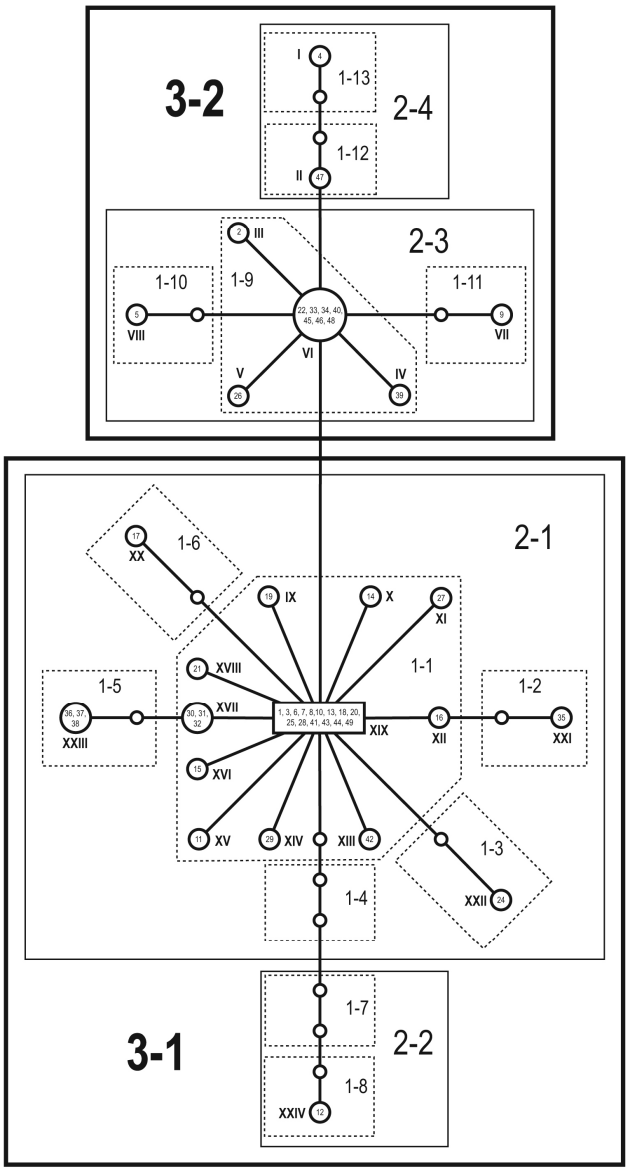
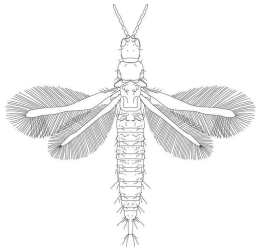


Figure 7: The mtDNA COI haplotype network cladogram of *K. nicolsoni* specialising on *A. papyrocarpa*. The lines connecting circles in the network represent single mutational changes. The small dots joining some of these lines indicate an interior clade that is inferred as an intermediate haplotype not present in the sample. Haplotype numbers are given in Table 1. The dotted-line polygons indicate the 1-step clades haplotypes, grouped together into 2-step clades (thin lined polygons), and the thick lines indicate the 2-step clades nested into 3-step clades. Sample numbers are indicated within the clade.



CHAPTER IV.

PERSISTENCE AND MAINTENANCE OF RESOURSE SHARING AMONGST GALL-THRIPS SPECIES

ABSTRACT

Niche overlap need not necessitate competitive exclusion where limited resources might be shared among species. Related species conceivably avoid competitive exclusion by differing in the manner by which they utilise a resource or whereby habitat heterogeneity and dispersal limitation mitigate negligible competitive interactions. However, there has been no direct evidence showing temporal scales of resource sharing of closely related specialists. Here we discuss the persistence of resource sharing over timescales perhaps much greater than those ecological, between closely related insect lineages that demonstrate negligible differences in their utilisation of nutrients, shelter, and space. We infer a phylogeny of pairs of lineages, whose extant progenitors share the same host, and generate a chronogram calibrated using palaeobotanic and palaeoclimatic evidence. The outcome of these inferences indicates that resource sharing among phytophagous animals could possibly persist for periods counted in millions of years. Colonisation limitation, predator/parasite-mediated competitive effects, and/or asymmetries between dispersal and competitive abilities are suggested as mechanisms that have possibly led to the collapse of niche partitions.

INTRODUCTION

There have been many observations of significant niche overlap between species and consequently models endeavouring to explain resource sharing and maintenance of species occupying similar niches have flourished (Volterra, 1926; Hutchinson, 1959; Hairston *et al*., 1960; Hardin, 1960; MacArthur & Levins, 1964 & 1967; Pianka, 1974; Chesson, 1994; Inbar & Wool, 1995; Webb *et al*., 2002; Palmer *et al*., 2003; Egas *et al*., 2004). How long might these associations persist? Differences in the way species utilise resources have traditionally been thought of in terms of interspecific competitive effects acting on the stability of such communities through time and space (Schoener, 1974). Transient and patchy resources have also been cited (Hanski, 1990; Tilman, 1994; Levin *et al*., 2001) as playing a part in how local-scale competitive interactions take place in spatially structured habitats.

Arid-adapted *Acacia* are host to a large diversity of thrips suites that invariably specialise on the plant or on one another (Morris *et al*., 2002). Of this group, the gall-inducing thrips comprise one ecological-behavioural suite whose diversity partly depends on a highly specialised affiliation with host *Acacia* species (Ananthakrishnan, 1992; Crespi *et al*., 1998; Morris *et al*., 1999). Gall-inducing *Acacia* thrips are a monophyletic taxon (Morris *et al*., 2001; Morris *et al*., 2002) implying that gall induction in this group has a single origin. The development and growth of galls has been widely correlated with the feeding activity and nutritional requirements of the galler (Mound, 1994; Karimi *et al*., 2000; Brodbeck *et al*., 2002; Nyman, 2002). Single females, or a female-male pair, induce a gall on the phyllode (leaf-like modified petiole) of a specific host plant (Crespi & Worobey, 1998) and become fully enclosed within a few days after initiation (Mound *et al*., 1996). Within the natal gall, eggs develop through two mobile larval stages, two or three quiescent pupal stages, and eclose as mobile adults (Crespi & Mound, 1997). Three host species, *Acacia papyrocarpa*, *Acacia microcephala*, and *Acacia tephrina*, each support a pair of putative species belonging to the *Kladothrips rugosus* complex. Each pair comprises two discrete gall morphologies that take a generalised form of either an elongate or pouched

(tending to spherical) structure (Figure 1). Within the elongate and pouched gall-types, appearance also varies by the presence/absence of either spiky protrusions or prominent longitudinal ridges.

Galls are paramount in providing food, shelter, and a vessel for reproduction. The extremely high value placed on this resource has apparently has resulted in competition for 'gall-space' (Crespi *et al*., 2004). Gall induction by founders is only permitted when new phyllode growth is available for inoculation. This event is usually dictated by local, infrequent, and unpredictable arid-zone rainfall patterns. Synchronicity of gall induction is believed to foster competition for suitable sites between foundresses before and after inoculation. Competition occurs among other gall-thrips specialising on the same host *Acacia* species and other usurping thrips species (Crespi, 1992*a* & *b*).

Pairs of sympatric gall-thrips species, each inducing either an elongate or pouched gall-type on the same host species, across different host lineages are either the result of cospeciation or convergence. Repeated patterns of gall-type pairs on different host species are present in a sister-clade supported by a more distantly related host, and has been inferred to occur across ancestral gall-thrips lineages (Crespi *et al*., 2004). This study investigates putative gall-thrips species that inhabit the same host species and explores links between phylogeny, species diversity, specialisation, and ecological interactions to develop hypotheses of how diversification has been driven by resource availability over evolutionary time scales. The aim of this work is to estimate an upper limit of the relative time of persistence between several pairs of insect species specialising on the same host species and discuss the ecological interactions defining this relationship.

METHODS

We approximate the age of key divergences using penalised likelihood and estimate the period of time where resourse sharing between sympatric species of the *K. rugosus* complex inhabiting the same host species might have

persisted. Evidence (Morris *et al*., 2002) indicates that the *K. rugosus* species complex comprises a series of host specific siblings. Some of the pairwise distances among the complex indicate below species level relationships. Other recent work has also identified a new species, *Kladothrips nicolsoni*, one of two sympatric species on *A. papyrocarpa* belonging to the *K. rugosus* complex (McLeish *et al*., 2006). The *K. rugosus* species complex, a monophyletic clade with a preponderance of emerging species (McLeish *et al*., 2007 in press) provides the best opportunity to assess the duration of resourse sharing by gall-thrips on *Acacia*. Vicariance, sympatry, dispersal (colonisation), and extinction processes (Levins, 1969; van Nouhuys & Hanski, 2002) cloud interpretations of phylogenetic inference. However, these processes become more transparent for taxa in an initial period of differentiation (Brown *et al*., 1995).

Phylogenetic analysis

We added sequence data of COI, EF-1a, 16S, and *wingless* gene fragments from 15 races of the *K. rugosus* species complex that specialised on 12 *Acacia* host species to a previously published gall-thrips phylogeny (Morris *et al*., 2001), which had only one representative of the complex. The four gene fragments comprised 2606 base pairs. *Rhopalothripoides* (Bagnall) was used as an outgroup as it is the most closely related sister-genera to *Kladothrips* (Froggatt) (Morris *et al*., 2002). Putative host-related species were pruned from the dataset to generate a chronogram that included only sympatric races from the *K. rugosus* complex as the reduced phylogeny provided a more suitable number of pseudoreplicate trees for confidence limit estimates (see below). Maximum parsimony was implemented in PAUP*b4.10 (Swofford, 2002) employing a heuristic search, with TBR (tree bisection-reconstruction), branch swapping on all best trees, with 500 random sequence additions holding 10 trees held at each step. We used 1000 heuristic pseudoreplicates to calculate bootstrap support values (Felsenstein, 1985), using the same search parameters as above for the pseudoreplicates.

Bayesian inferences were generated under priors given in McLeish *et al*. (2006) and implemented in MrBayes (MrBayes 3.0b4, Huelsenbeck & Ronquist, 2001). To accommodate differences in substitution rate parameters

and heterogeneity of base composition in our multiple gene fragment dataset we fitted separate models to six partitions comprising 1st, 2nd, and 3rd codon positions of the *cytochrome oxidase one* (COI) mitochondrial data, with single partitions for each of *elongation factor one alpha* (EF-1α), *wingless*, and the 16S gene fragments. We used a general time reversible (GTR) DNA substitution model with gamma distributed rates with a proportion of invariant sites. Posterior probabilities and mean branch lengths were derived from 3000 trees taken from post-burnin generations 3.5-5.0 million, after the chains had reached apparent stationarity, sampling every 500th generation. We ran the Bayesian analysis 3 times to verify the repeatability of the phylogenetic outcome.

Construction of chronograms

Semi-parametric penalised likelihood (PL) rate smoothing with a truncated Newton algorithm was implemented to approximate the relative age of divergences at key nodes using r8s (Sanderson, 2003). To generate a PL chronogram, we used MrBayes to generate a consensus phylogeny using the same priors as described above. Modeltest 3.0 (Posada & Crandall, 1998) was used to determine the appropriate nucleotide substitution model (GTR + I + G) and gamma shape required for r8s analysis. The outgroup *Rhopalthripoides* was pruned prior to analyses using r8s. We filtered post-burnin trees having identical topology from our Bayesian analysis to generate the 95% central distribution intervals for node-age estimates. These 685 identical trees from the MCMC run were filtered trees and chronograms generated for each. The resulting branch length estimates were used to calculate the ages of nodes for each tree. The 2.5% right and left tails were then removed, providing 95% central distribution limits which were used as a measure of confidence for the nominated node estimates (Schwarz *et al*., 2006).

Divergence events were calibrated using constrained maximum and minimum times at the root node and an internal node (see Chapter III p. 68). The appearance of fossil *Acacia* pollen in Australia 25 mya (Truswell & Harris, 1982; Martin, 1994) and the distribution of the *Acacia* subgenera *Phyllodineae* hosts to which the gall thrips are confined, indicate that

radiations of this group occurred in Australia no more than 15 mya (Truswell & Harris, 1982; Maslin *et al*., 2003). Given the appearance of gall-thrips hosts presumably coincided with the appearance of arid-adapted *Acacia* during the Miocene we constrained the root node to 10-15 mya. The host group of *Acacia* subgenera *Plurinerves* on which *K. rugosus* specialise experienced a rapid period of speciation over the Quaternary (Maslin & Hopper, 1982; Byrne *et al*., 2002; Andrew *et al*., 2003; Miller *et al*., 2003). Therefore, the common ancestor of the *K. rugosus* species complex was constrained to between 2-5 mya.

RESULTS

Phylogenetic analysis

Maximum parsimony and Bayesian phylogenetic analyses of the larger taxa set are more or less congruent with one another. Support for monophyly was very strong at the inferred origin of the *K. rugosus* species complex (Figures 2 & 3). Weak support at some bifurcations and below species level uncorrected "p" distances for COI between the more derived *K. rugosus* taxa (see Appendix IV) suggest sufficient divergence has not occurred to better resolve these relationships.

Lack of resolution among comparable lineages in the Bayesian consensus tree (Figure 3) used to generate the chronogram parallels those evident in our larger taxa set phylogenies (Figures 2 & 3). This lack of support is believed to indicate a hard molecular polytomy suggesting rapid speciation events over a short period (Chapter III). There was a high level of confidence at the root node and the common ancestor of the *K. rugosus* complex, and these were used as calibration points to estimate node ages.

Chronogram

We used a gamma shape distribution value of 0.6812 to estimate the age at the root and at the common ancestor of the *K. rugosus* complex. The cross validation analysis yielded a smoothing value of 34.67. Penalised likelihood

analysis using our Bayesian consensus inference of the reduced taxa set (Figure 4) indicated that diversification of the *K. rugosus* complex began between 2.91 and 4.99 mya. This age represents an upper limit for resourse sharing of species inhabiting the same host species assuming extinction, colonisation, and vicariance events have been negligible. The age of the root of the phylogeny was dated at between 10.00 and 14.17 mya.

DISCUSSION

Relative age estimates indicate the co-occurrence of lineages belonging to the *K. rugosus* species complex might have persisted for up to 3 million years, and therefore, species sympatry potentially existed in tandem over timescales greater than those ecological. Gall-thrips exhibit almost identical resource use, local-scale phenology (dispersal and gall induction), and mouthpart morphology (nutritional acquisition). This contrasts life history variation and gall structure polymorphisms for each gall-thrips species pair that share a single host species. The long-term persistence of these sympatric species can be explained in terms of recruitment limitation or divergent natural selection on traits impacting resource utilisation.

Patchy host distributions provide a heterogeneous resource. Harsh arid conditions imply that sites suitable for successful colonisations are ephemeral. The extent to which galling sites represent a limited resource is not fully appreciated (Crespi *et al*., 2004). Resource availability would presumably be higher for a disperser arriving (perhaps by chance) at a distant host patch than dispersing to another site on the natal host plant. The only evidence for dispersal behaviour suggests inter-seasonal movement between the natal gall and novel colonisation sites extends to distances of at least 1 km, and this is compatible with between host-patch migrations (McLeish *et al*., 2003). Therefore, spatial heterogeneity of host species has important ramifications for competitive interactions where recruitment and environmental stress play a key role (Menage & Sutherland, 1987; Hubbell *et al*., 1999).

The ability of an organism to disperse can impose particular ecological circumstances (competition and predation) on species that consume transient and patchy resources (Tilman, 1994). Motile species arriving at a colonisation site can be subject to recruitment limitation, important for shaping local successional-like interactions. That is, species growth and survival is limited by the inability to recruit at all favourable sites (Hubbell *et al*., 1999). If recruitment is low for gall-thrips arriving at a favourable site, then competition for space is minor or insignificant amongst species.

The harsh environmental conditions presented by the arid-zone might reduce predation, allowing competition to be more prevalent in shaping the community in a newly available habitat (Menage & Sutherland, 1987). Sympatric species utilising a common resource potentially can coexist when competitive ability is asymmetrical between competitors, where the poorer competitor is a better disperser (Tilman, 1994; Amarasekare & Nisbet, 2001).

Host plant specialisation by gall-thrips reveals predictable relationships between pairs of gall-thrips species each bearing one spherical gall and one elongate gall (Crespi *et al*., 2004). Gall morphology variation within the *K. rugosus* complex is extreme by comparison to other gall-thrips species (Crespi & Worobey, 1998). The spherical galls have a smaller surface area to volume ratio and provide space for the relatively large brood sizes observed in spherical galls of other species. The elongate galls have by contrast a smaller relative volume, and this is reflected by the smaller brood sizes of species with galls of this structure. Gall sphericity is associated with physogastry (Crespi & Worobey, 1998) that is associated with high-fecundity. Sphericity of galls enables larger brood sizes and this has been associated with a boom-bust life history strategy. Resource sharing might have conceivably emerged via divergent natural selection on gall polymorphisms (Crespi & Worobey, 1998) and/or life history variation.

Exploitative suites of thrips genera are also known to specialise on gall-thrips species and the gall structure might be a selective response to natural enemies such as those from the genus *Koptothrips* (Crespi & Worobey, 1998; Crespi & Abbott, 1999; Morris *et al*., 2002). Evidence suggests that spatially heterogeneity and predator/parasite pressure may or may not drive sympatric

diversification (Doebeli & Dieckmann, 2000; Brockhurst *et al*., 2003) though increased niche overlap within a group of species has been empirically shown to alleviate net competitive effects from species outside the group (Pianka, 1974; Krause *et al*., 2003).

It is generally accepted that species diversity is partly determined by the partitioning of available resources. Violation of the competitive exclusion principle (Hardin, 1960) has not been demonstrated directly under the premise of the absolute time of persistence of resourse sharing. Species that have similar ecologies and occupy the same spatial region over enormous timescales might share resources by differences in life history strategies that permit a net reduction in competitive interactions or can tolerate thresholds of fluctuations in recruitment densities.

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Figure 1: A photograph of sympatric gall-thrips populations (putative species) inhabiting *Acacia tephrina* that induce dramatically different gall-types with pouched-spiky and elongate morphologies. Inset: the ridged gall-type induced by one of the sympatric gall-thrips populations on *Acacia papyrocarpa*.

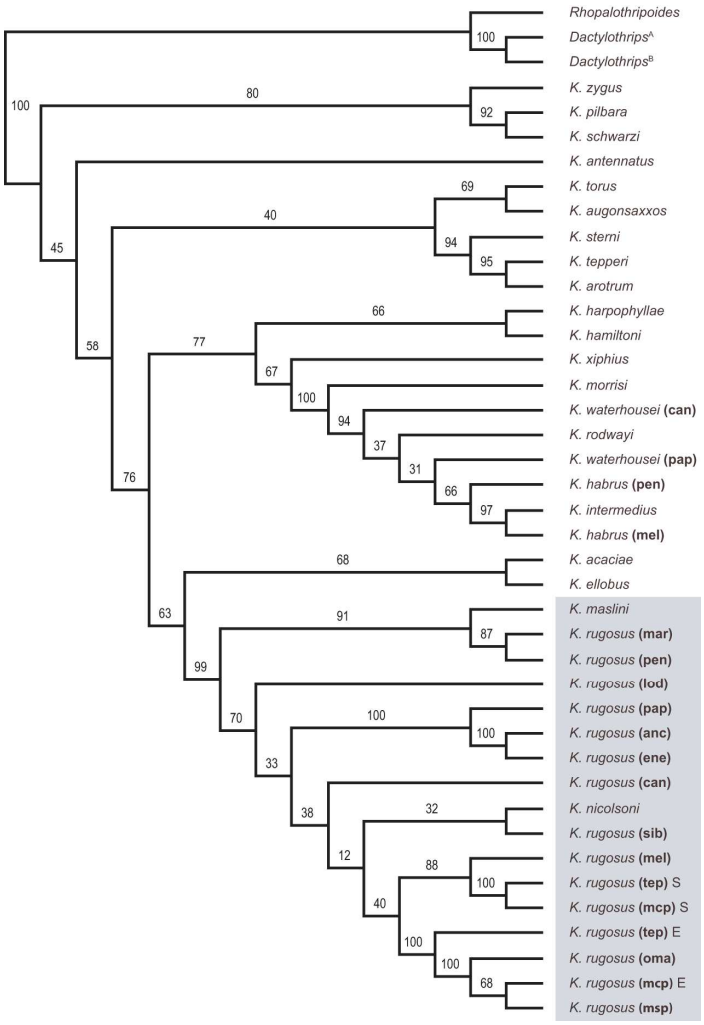


Figure 2: A maximum parsimony phylogeny is shown and was constructed using COI, EF-1α, *wingless*, and 16S genes. A heuristic search with tree bisection-reconstruction (TBR) branchswapping, random addition of taxa 500 replicates per search and 10 trees held at each step), and 1000 bootstrap replicates was implemented. The *K. rugosus* species complex is highlighted with a grey box. Host tree species are abbreviated in brackets as follows: *A. ammophila* (amm), *A. ancistrophylla* (anc), *A. cana* (can), *A. enervia* (ene), *A. loderi* (lod), *A. maranoensis* (mar), *A. melvillei* (mel), *A. microcephala* (mcp), *A. microsperma* (msp), *A. omalophylla* (oma), *A. oswaldii* (osw), *A. papyrocarpa* (pap), *A. pendula* (pen), *A. sibilans*, and *A. tephrina* (tep). Taxon codes are as follows: E = elongate and S = spiky-spheroid gall structures.

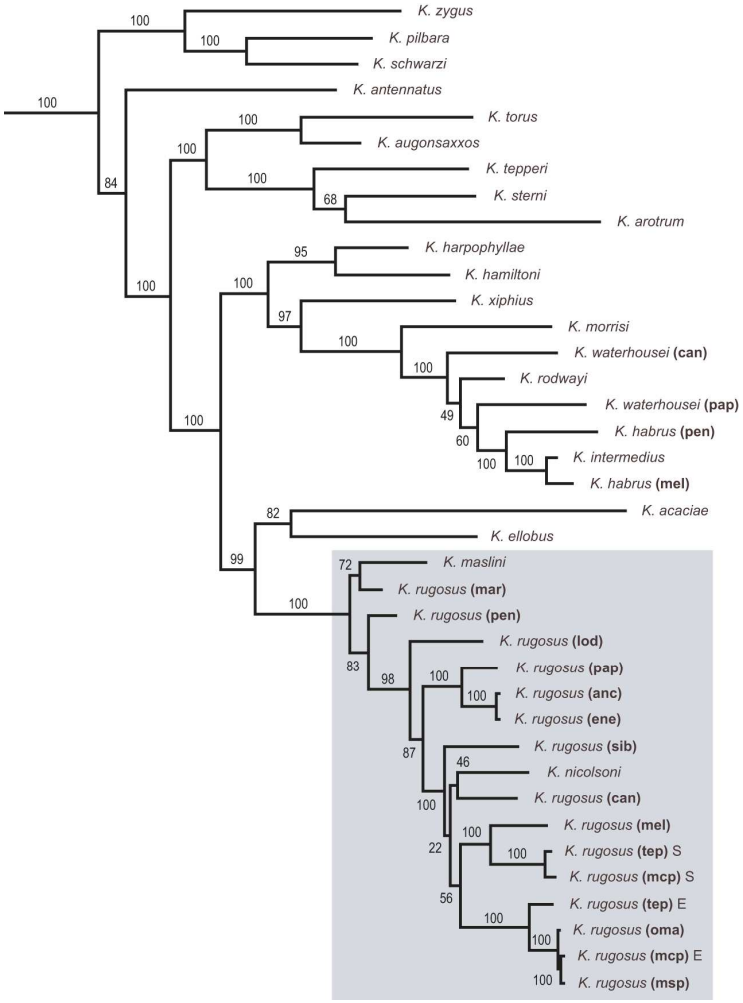


Figure 3: This Bayesian Consensus phylogram was inferred using six separately modelled partitions comprising 1st, 2nd, and 3rd COI codons and separate EF-1α, *wingless*, and 16S sites. Posterior probabilities and branch lengths are derived from 3000 trees taken from a sample of 5 million generations, sampling every 500th generation. The *K. rugosus* species complex is highlighted with a grey box. Abbreviations for *Acacia* host races are as follows: *A. ammophila* (amm), *A. ancistrophylla* (anc), *A. cana* (can), *A. enervia* (ene), *A. loderi* (lod), *A. maranoensis* (mar), *A. melvillei* (mel), *A. microcephala* (mcp), *A. microsperma* (msp), *A. omalophylla* (oma), *A. oswaldii* (osw), *A. papyrocarpa* (pap), *A. pendula* (pen), *A. sibilans*, and *A. tephrina* (tep). Taxon codes are as follows: E = elongate and S = spiky-spheroid gall structures.

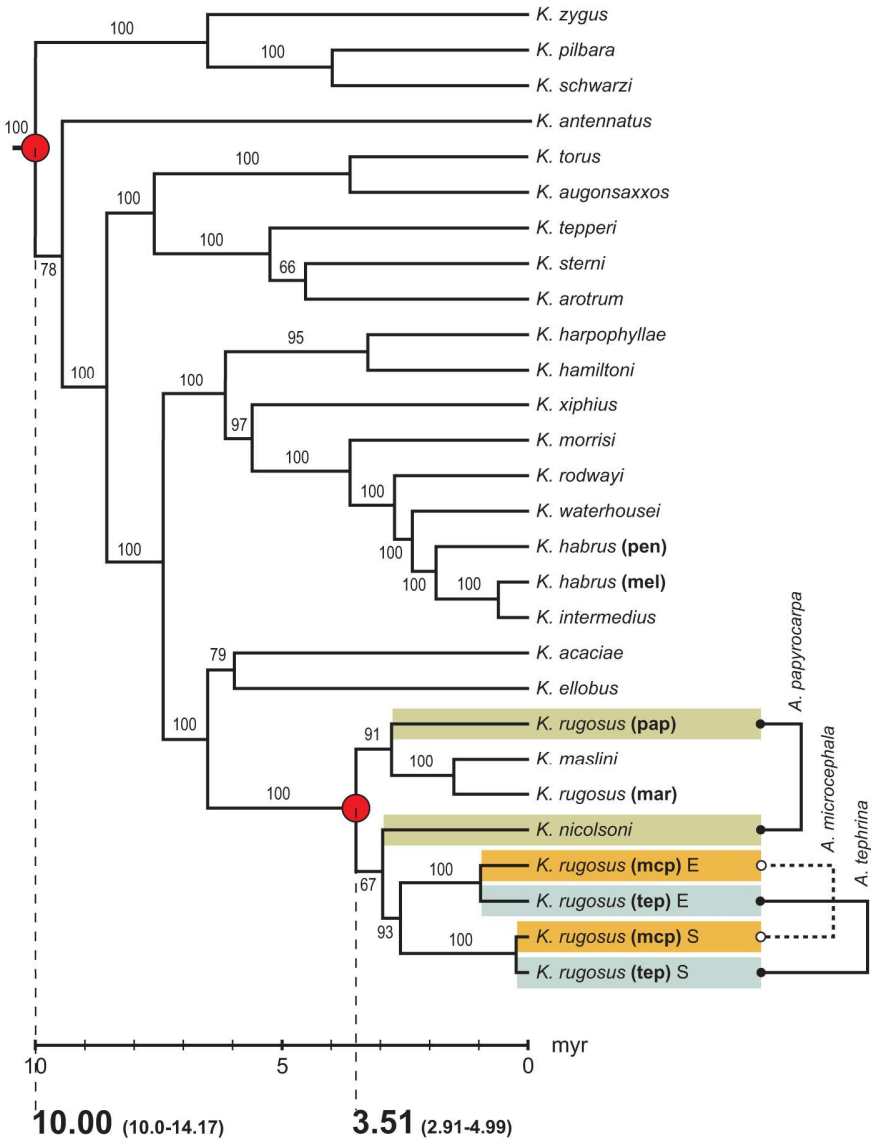
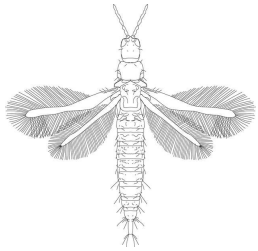


Figure 4: A penalised likelihood chronogram of our Bayesian consensus tree. Posterior probabilities and branch lengths are derived from 3000 trees taken from a sample of 5 million generations, sampling every 500th generation. Colour rectangles highlight lineages that terminate in taxa that specialise on a common host species: *A. papyrocarpa* (pap), *A. microcephala* (mcp), and *A. tephrina* (tep). Open node circles show constrained calibration points between 10-15 mya and 2-5 mya. Taxon codes are as follows: *A. pendula* (pen); *A. melvillei* (mel); E = elongate; S = spiky-spheroid.



GENERAL CONCLUSION

Phylogenetic inferences of this group comprising emerging species has captured taxa at various stages of differentiation in the continuum to species thus providing a snapshot of probable pathways to speciation available to these phytophagous insects. Diversification here appears to have arisen via several non-mutually exclusive mechanisms. Generally, the transition from host preference to host race is followed by a transition from host race to reproductively isolated species.

A non-independent phylogenetic association between the *Kladothrips rugosus* and *Kladothrips waterhousei* species complexes was detected. Diversification in phytophagous insects can proceed as a result of synchronous divergence episodes and to independent modes of speciation in both insect and host lineages. In addition to cospeciation and host switching, host *Acacia* response to climatic and geographic effects in expanding arid regions of Australia appears to have given rise to range expansion and allopatric differentiation among populations and implies gall-thrips diversification is passively linked to host evolution. Opportunity for cospeciation and host switching should increase under heightened rates of host diversification. Speciation in gall-inducing *Acacia* thrips might ensue by the formation of host-related races via cospeciation or host switching where there is reduced gene flow among populations of a single species parasitising two or more host populations, eventually leading to reproductive isolation between them (for example: Jaenike, 1981; Jaenike, 1990; Emelianov *et al*., 1995; Parsons & Shaw, 2001; Drés & Mallet, 2002). Selection for the evolution of host and ecological specialisation in diet and habitat use, and therefore a potential for reproductive isolation between sympatric groups, is consistent with the supposition of life history tradeoffs between sympatric gall-thrips morpho-types sharing the same resource. Furthermore, the level of host specificity in gall-thrips might be partly determined by the ability to disperse. Host specificity may arise as a result of poor vagility and very low frequencies of successful dispersal events among novel host species.

The relationship between gall-thrips and *Acacia* is presumed to be evolutionarily conservative because of preferences for galling sites necessary for brood-laying and brood development. High host specificity attests to the fidelity of this relationship (Ananthakrishnan, 1992) where the gall, as an extended phenotype (Crespi & Worobey, 1998), can differ markedly among closely related sister-taxa. Host races are maintained by reduced gene flow because of differential host preference. In addition to host preference, the following criteria are satisfied in at least several instances: breeding individuals of sympatric gall-thrips host races (morpho-types) are within dispersal range of one another; and reduced gene flow among populations is significant.

Gall-thrips show preference for a group of closely-related *Acacia* hosts (at least lower than subgeneric). This affiliation is tied inseparably to brood-laying, diet, and possibly for mating sites (Jaenike, 1990; Craig *et al*., 2001). However, it is less likely that differential selective pressures acting on phenological differences between hosts (Butlin, 1990; Emelianov *et al*., 1995) and predation risk associated with various *Acacia* (Crespi & Sandoval, 2000; Mira & Bernays, 2002) are strong among taxonomically close hosts (McCoy *et al*., 2001). Selection for specialisation and shifts to new host species should necessitate unique adaptations, and therefore, evolve to be highly responsive to host variation. This appears to be consistent with the degree of the behavioural and morphological shift observed in successful host switches inferred for gall-thrips as being a function of the (phenological, chemical, and genetic) barriers overcome by the colonisation. An inferred host switch by an ancestral gall-thrips species to a novel host might have been accompanied by a decrease in predation pressure or mortality associated with the natal host (Futuyma & Moreno, 1988; Mira & Bernays, 2002) and might partly be responsible for subsequent range expansion in tandem with host range expansion and an increase in niche breadth. This work reinforces the importance of multiple factors contributing to insect-plant affiliations.

Coevolution between gall-thrips and *Acacia* can be summarised in general terms as joint speciation (cospeciation) of interacting lineages (Herre *et al*., 1996) and as evolutionary change in one species followed by an evolutionary response in the other species (Janzen, 1980) over a relatively long

period of time. Gall-thrips appear to adhere to a level of host-tracking and this has been shown at higher (Crespi *et al*., 2004) and now lower taxonomic levels and this is in contrast to most phytophagous insects that do not cospeciate with their host plants (Futuyma, 2000). Ehrlich and Raven (1964) assumed a reciprocal selection between insects and plants leads to diversification of chemical resistance in plants and food specialisation in insects. It is still not clear whether the association between gall-thrips and *Acacia* host-plants are antagonistic interactions or not but is highly plausible for this system. Direct testing of such an interaction between thrips and *Acacia* remains for future work.

Describing taxonomic relationships among the *Kladothrips rugosus* species complex remains. Slight morphological differences have been shown between *Kladothrips nicolsoni* and the 'ridged' gall-morph of *K. rugosus* on *Acacia papyrocarpa*. Other sympatric pairs having striking disparities in gall structure might indicate two species complexes, one with rugose galls and the other having the elongate gall-type. Future work needs to be directed at elucidation of interactions between gall-thrips and *Acacia*. Comparative and experimental approaches can be used to exploit the sound phylogenetic relationships already established to test hypotheses of evolution of gall-thrips resistance and the *Acacias* tolerance to herbivory.

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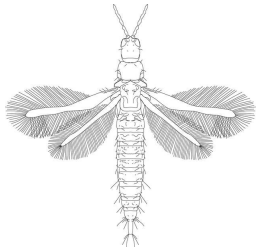


APPENDIX I

Photographic images of Australian gall-inducing *Acacia* thrips.

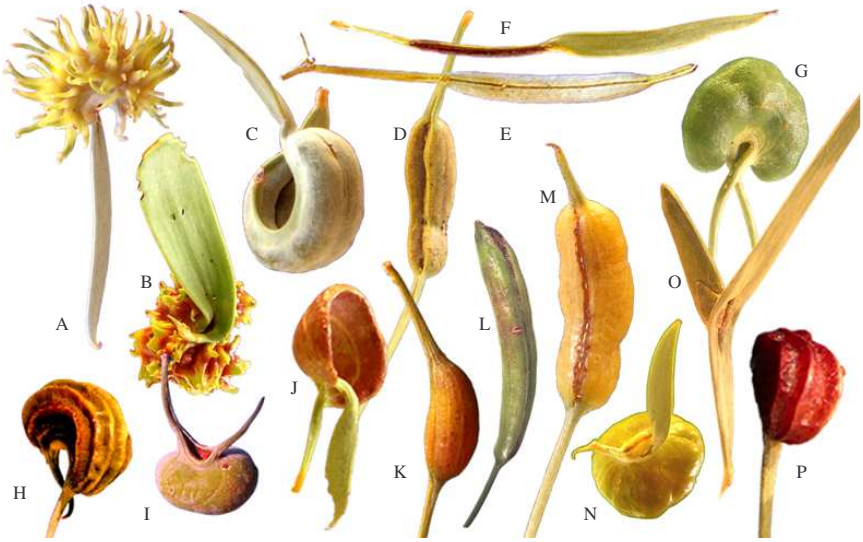


Figure 1: Interspecific gall-form variation induced by gall-inducing thrips that specialise on Australian *Acacia*. Each gall represents a type from a specific *Acacia* (not to scale). The gall-thrips species and host are indicated by the following letters: A = *Kladothrips rugosus* on *Acacia tephrina*; B = *K. rugosus* on *Acacia melvillei*; C = *K. rugosus* on *Acacia tephrina*; D = *K. rugosus* on *Acacia microsperma*; E = *Kladothrips morrisi* on *Acacia calcicoli*; F = *Kladothrips hamiltoni* on *Acacia cambagei*; G = *Kladothrips sterni* on *Acacia aneura*; H = *K. rugosus* on *Acacia papyrocarpa*; I = *Kladothrips arotrum* on *A. aneura*; J = *Kladothrips ellobus* on *A. cambagei*; K = *Kladothrips nicolsoni* on *A. papyrocarpa*; L = *K. rugosus* on *Acacia microsephala*; M = *K. rugosus* on *Acacai loderi*; N = *K. rugosus* on *Acacia pendula*; O = *Kladothrips maslini* on *Acacia orites*; and P = *K. rugosus* on *A. papyrocarpa*.

A.

B.

Figure 2: A cross section of galls induced by (A) *Kladothrips sterni* that is found on *Acacia aneura* and is the only know gall-thrips species in this group that induces a chambered gall, and (B) *Kladothrips rugosus* that inhabits *Acacia melvillei*.

APPENDIX II

The substitution rates and base composition bias for *cytochrome oxidase I* (COI), *elongation factor alpha I* (EF-1α), *wingless*, and 16S (ribosomal RNA subunit) gene fragments isolated from gall-inducing thrips taxa are explored.

Base composition, transformation rates, and saturation

Heterogeneous base compositions among different gene regions can lead to high levels of homoplasy problematic to correctly identifying useful phylogenetic signal. Several recent studies have provided direction for modelling substitution and rate dynamics of marker genes used in this study (Lin & Danforth, 2004; Schwarz *et al*., 2004). We evaluated our data mindful of the following considerations taking the following approach. Mitochondrial genes have been show to have strong A-T bias in insect genomes particularly for third codon positions compared with nuclear genes that have a more uniform base composition. Base composition for each codon position was measured separately for each gene region with PAUP* (Swofford, 2002) using Chi-square analysis to test variation among taxa. Different substitution rates among sites and genes can lead to the underestimation of change along lineages. We used MrBayes 3.0b4 (Huelsenbeck & Ronquist, 2001) to calculate transition and transversion rates for COI 1^{st}, 2^{nd}, and 3^{rd} codon partitions, EF-1α, *wingless*, and 16S gene fragments to investigate substitution dynamics.

Phylogenetic signal is potentially interfered with by multiple substitutions at any given site (Schwarz *et al*., 2004). To test for saturation, codon positions of COI and EF-1α fragments were partitioned to estimate the most appropriate model of evolution using Modeltest 3.0 (Posada & Crandall, 1998). Pairwise distances were generated using the maximum likelihood model estimated by Modeltest for each partitioned codon. The mean values (±2 SE) of both 1^{st} and then 3^{rd} position distance estimates were plotted against categorised 2^{nd} position distances (Figures 1-2). Deviations from linearity are indicative of homoplasious effects on branch length and sequence divergence estimates. Conversely, a linear relationship between distances from any two partitions would be expected where each estimates equivalent distances.

The utility of 16S as a suitable marker is brought into question due to high A-T richness and substantial site-to-site variation in substitution rate. The software program *Mfold* (Zuker, 2003; Y-Ding *et al*., 2004) was used to estimate loop and stem regions in our 16S fragment. The program generated a considerable number of plausible but highly variable rRNA structures based on

lowest free energy state estimates. The GTR model (Yang, 1994) of sequence change was used to provide the best correction for A-T bias in 16S rRNA as it simultaneously considers site-to-site variation and compositional bias allowing A-T transversions to be treated independently (Whitfield & Cameron, 1998). In addition, we used ClustalX (Thompson *et al*., 1997: available from http://www.embl.de/~chenna/clustal/darwin/) to identify and remove ambiguous alignments in our 16S dataset and implemented a model parameter in our MrBayes analysis that assumes autocorrelation amongst adjacent nucleotide positions.

Analyses outcomes

Chi-square tests for differences in base composition bias among taxa (Table 1) indicated no significant differences for any of our partitions although the COI 3rd codon positions showed a relatively high A-T bias. Graphical plots between pairwise maximum likelihood distances (Figures 1a & 1b) showed a non-linear relationship between COI 3rd codon mean distances and 2nd codon category distances indicating saturation effects for 3rd codon positions. The plot for COI 1st distances does not show as radical deviation from linearity. Plots for EF-1α 3rd positions do not indicate a violation of linearity that would indicate saturation problems. Bayesian estimates for GTR substitution rate matrices, proportion of invariant sites, and γ–shapes (Table 2) indicate differentiation in evolutionary rate determinants among partitions. Substitution matrices showed heightened A-G rates at COI 3rd position and C-T rates at COI 1st and 3rd positions. Differential rates among COI codons, EF-1α, *wingless*, and 16S genes support our decision to partition the multi-gene dataset.

Table 1: Mean base frequencies and χ^2 tests of bias for codon positions of COI and EF-1α, and for *wingless* and 16S gene fragments. Third codon position COI A-T bias highlighted in bold.

Codon	A	C	G	T	**%A-T**	χ^2	***P***
COI							
1st	0.31808	0.10893	0.23353	0.33946	65.75	81.22 $_{df=168}$	1.0
2nd	0.18010	0.20659	0.16282	0.45049	63.06	56.65 $_{df=168}$	1.0
3rd	0.38657	0.07957	0.04169	0.49216	**87.87**	188.22 $_{df=135}$	**0.13**
EF-1α							
1st	0.30860	0.17843	0.40107	0.11190	42.05	8.93 $_{df=135}$	1.0
2nd	0.33288	0.27500	0.14598	0.24613	57.90	21.12 $_{df=135}$	1.0
3rd	0.20933	0.32320	0.12343	0.34404	55.34	30.92 $_{df=135}$	1.0
Wingless	0.28694	0.28916	0.22605	0.19784	48.48	91.11 $_{df=135}$	1.0
16S	0.42133	0.09620	0.12437	0.35810	77.94	76.82 $_{df=168}$	1.0

Table 2: Substitution rate matrices, proportion of invariant sites (*p*(inv)), and γ-shapes (α), estimated using Bayesian analysis of 1st, 2nd, and 3rd COI partitions and for EF-1α, *wingless*, and 16S gene fragments.

Codon position	C	G	T	*p*(inv)	α
1st position COI					
A	4.97364	5.77258	0.98654	0.53012	0.92991
C		0.35808	**46.29341**		
G			1		
2nd position COI					
A	1.15293	2.07373	1.53047	0.79176	8.19798
C		0.75108	2.97773		
G			1		
3rd position COI					
A	1.24894	12.56477	0.12101	0.01098	1.74892
C		1.11355	**22.44448**		
G			1		
EF-1α					
A	1.97336	2.88465	2.49349	0.45248	6.37401
C		1.07735	10.61902		
G			1		
wingless					
A	0.78979	1.97659	0.49217	0.41986	19.1542
C		0.74668	4.37042		
G			1		
16S					
A	1.158556	**16.122357**	2.27897	0.81636	0.29261
C		3.47678	**16.64657**		
G			1		

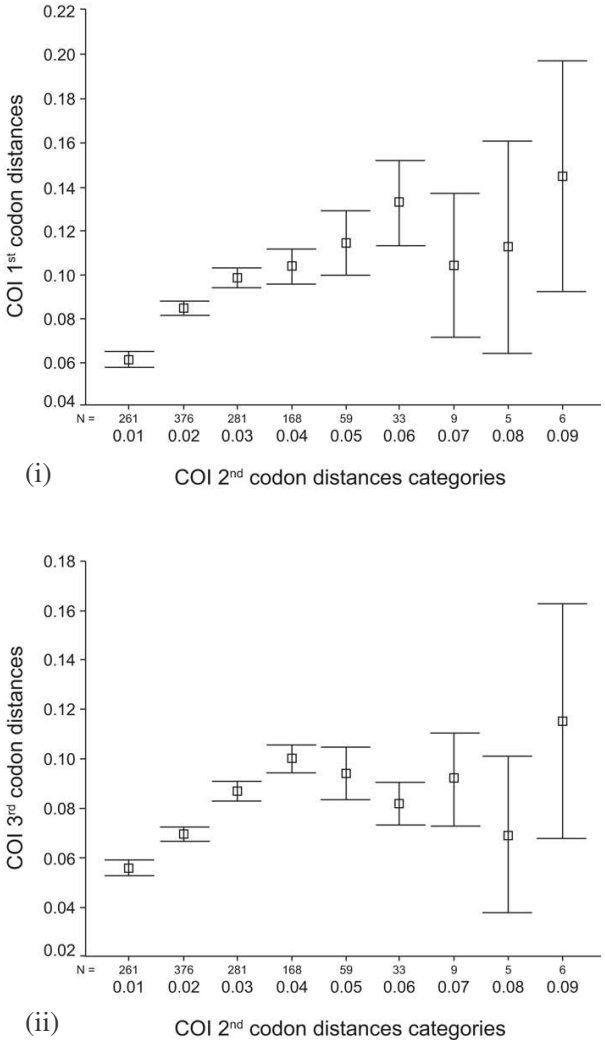
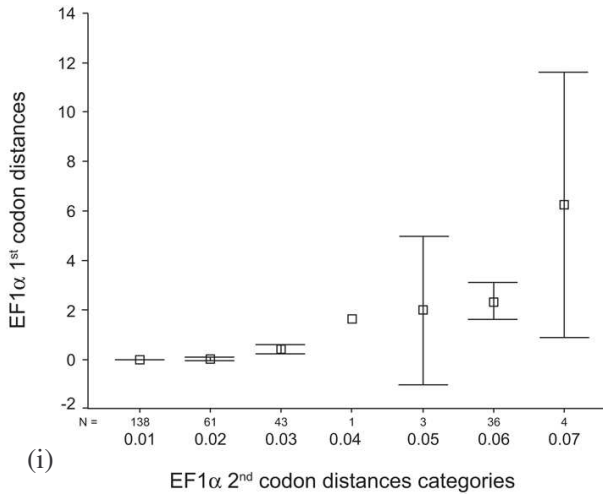
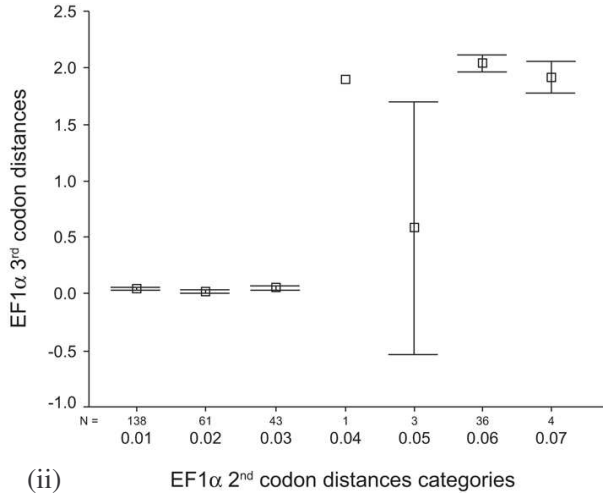


Figure 1: (i) Plots of mean maximum likelihood (ML) distances (±2 SE) generated from a model fitted to 1st COI codon positions against categorised distances for 2nd COI codon positions calculated using a separately fitted ML model. (ii) Plots of mean ML distances (±2 SE) generated from a model fitted to 3rd COI codon positions against categorised distances for 2nd COI codon positions calculated using a separately fitted ML model.



(i)



(ii)

Figure 2: (i) Plots of mean maximum likelihood (ML) distances (±2 SE) generated from a model fitted to 1st EF-1α codon positions against categorised distances for 2nd EF-1α codon positions calculated using a separately fitted ML model. (ii) Plots of mean ML distances (±2 SE) generated from a model fitted to 3rd EF-1α codon positions against categorised distances for 2nd EF-1α codon positions calculated using a separately fitted ML model.

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APPENDIX III

Comparisons between phylogenetic uncertainties generated by Bayesian analyses using either separate or partitioned DNA sequence data are explored.

Combined versus separate phylogenetic analyses

To accommodate differences in substitution rate parameters and heterogeneity of base composition in a multiple gene fragment dataset, it has been recommended that separate models be fitted to predetermined partitions in a dataset (Lin & Danforth, 2004) and this can be accomplished using a Bayesian approach. To test the benefits of partitioning the gall-inducing thrips DNA sequence data, support values generated by separate and combined analyses were contrasted (Brandley *et al*., 2005). The sequence data was either combined or divided into six partitions comprising 1st, 2nd, and 3rd codon positions of the *cytochrome oxidase one* (COI) mitochondrial data, with single partitions for each of *elongation factor one alpha* (EF-1α), *wingless*, and the 16S gene fragments. Separate models were fitted to each of six gene partitions or a single model to non-partitioned data in the Bayesian analyses and these two approaches were compared. A general time reversible (GTR) DNA substitution model with gamma distributed rates with a proportion of invariant sites was used in both instances. Posterior probabilities and mean branch lengths were derived from 3000 trees taken from generations 3.5-5.0 million, sampling every 500th generation. The sampled trees were derived from post-burnin generations after the chains had reached apparent stationarity.

Analyses outcomes

Bayesian inferences generated using a combined sequence dataset showed comparable levels of support when compared to the analysis using partitioned sequence data (Figure 1) and identical topologies (compare with Figure 2, Chapter III p. 99). Comparisons of phylogenetic uncertainty between the separate and combined analyses for each of the *Kladothrips rugosus* and *Kladothrips waterhousei* species complexes indicate negligible differences in support values (Figures 2-3).

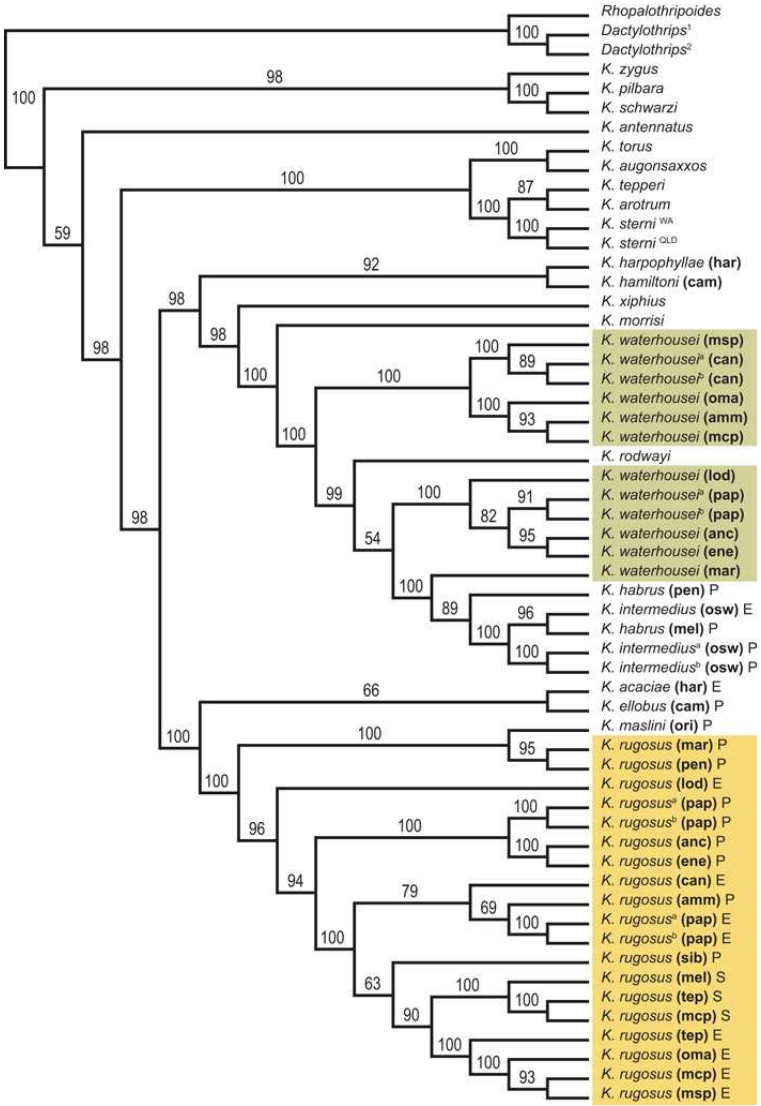

Figure 1: Bayesian majority rule consensus phylogenies of the *K. rugosus* species complex inferred from analysing combined sequence data. The combined analysis comprised no partitioning of the sequence dataset. A general time reversible (GTR) DNA substitution model with gamma distributed rates with a proportion of invariant sites was used. Posterior probabilities and mean branch lengths are derived from 3000 trees taken from generations 1.5-3.0 million, sampling every 500th generation. The sampled trees were derived from post-burnin generations after the chains had reached apparent stationarity. The coloured boxes indicate the *K. rugosus* and *K. waterhousei* species complexes. See page 57 for abbreviations & taxon codes.

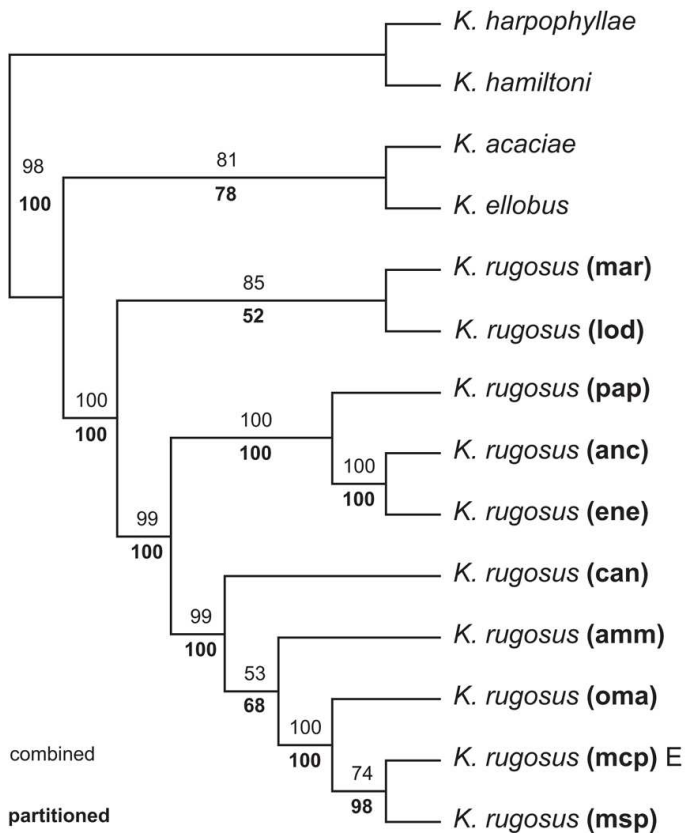

Figure 2: Comparison of Bayesian majority rule consensus phylogenies of the *K. rugosus* species complex inferred from either partitioned or combined sequence data. For the partitioned analysis, separate models were fitted to different gene partitions divided into six comprising 1st, 2nd, and 3rd codon positions of COI, with single partitions for each of EF-1α, *wingless*, and the 16S gene fragments. The combined analysis comprised no partitioning of the DNA sequence dataset. A general time reversible (GTR) DNA substitution model with gamma distributed rates with a proportion of invariant sites was used. Posterior probabilities and mean branch lengths are derived from 3000 trees taken from generations 1.5-3.0 million, sampling every 500th generation. The sampled trees were derived from post-burnin generations after the chains had reached apparent stationarity. Partitioned node support values are shown in bold below the branch. See page 57 for abbreviations & taxon codes.

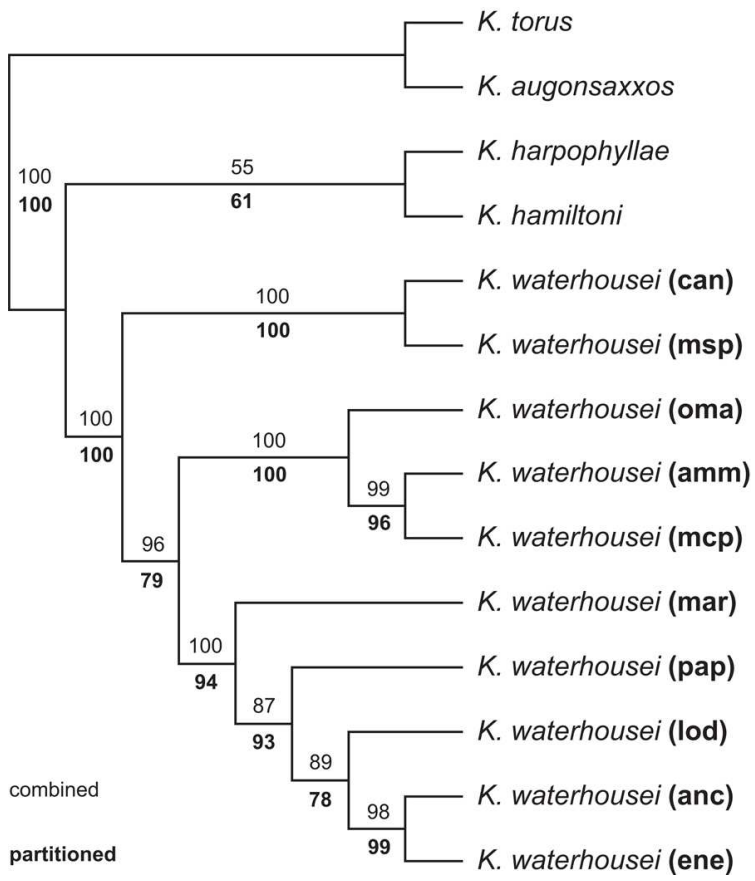

Figure 3: Comparison of Bayesian majority rule consensus phylogenies of the *K. waterhousei* species complex inferred from either partitioned or combined sequence data. For the partitioned analysis, separate models were fitted to different gene partitions divided into six comprising 1st, 2nd, and 3rd codon positions of COI, with single partitions for each of EF-1α, *wingless*, and the 16S gene fragments. The combined analysis comprised no partitioning of the DNA sequence dataset. A general time reversible (GTR) DNA substitution model with gamma distributed rates with a proportion of invariant sites was used. Posterior probabilities and mean branch lengths are derived from 3000 trees taken from generations 1.5-3.0 million, sampling every 500th generation. The sampled trees were derived from post-burnin generations after the chains had reached apparent stationarity. Partitioned node support values are shown in bold below the branch. See page 57 for abbreviations & taxon codes.

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APPENDIX IV

Genetic pairwise uncorrected “p” distances among the *Kladothrips rugosus* and among the *Kladothrips waterhousei* species complexes are calculated.

Genetic distances among complex taxa

Uncorrected "p" distances for the COI gene fragment indicated distances among the *Kladothrips rugosus* and *Kladothrips waterhousei* complexes (Tables 1-2) were generally compatible with species-level genetic differentiation shown among described gall-thrips taxa (Crespi *et al*., 2004). Species-level distances among gall-thrips are well in excess of 4.0% and up to 12% intergeneric distances. *Kladothrips habrus* sampled from *Acacia pendula* and another sample from *Acacia melvillei*, each inducing a similar elongate gall-type, did not group as sister-taxa with a COI uncorrected "p" distance of 6.0% between them (Table 3). A *Kladothrips habrus* did cluster with the *Kladothrips intermedius* sample with the same gall-type (Table 3). The *K. intermedius* populations sampled from the same host species, but each inducing a discrete gall-type, did not cluster with an uncorrected "p" distance of 8.0% between them (Table 3). The morphological difference between each of these *K. intermedius* samples was considered negligible (personal communication, Mound LA). The *Kladothrips sterni* populations from WA and QLD group as sister-taxa with a high level of support although branch length estimates indicate genetic divergences consistent with allopatric factors. Samples of *K. rugosus* and *K. waterhousei* on *Acacia papyrocarpa* and *K. waterhousei* on *Acacia cana* were replicated over time and space to test intra-taxon genetic variability. Each of the replicated populations unequivocally clustered as sister-taxa.

Table 1: Pairwise uncorrected "p" distances for 1245bp of COI mtDNA among *K. rugosus* populations found on *A. ammophila* (amm), *A. ancistrophylla* (anc), *A. cana* (can), *A. enervia* (ene), *A. loderi* (lod), *A. maranoensis* (mar), *A. melvillei* (mel), *A. microcephala* (mcp), *A. microsperma* (msp), *A. omalophylla* (oma), *A. papyrocarpa*, and *A. tephrina* (tep). Different *K. rugosus* gall structure abbreviations are given: spiky, elongate (elong.), and pouched (pouch.). *K. waterhousei* gall structures are uniform. Outgroup distances given by comparison with *Kladothrips* sister-genus *Rhopalothripoides* (Rha). Suspected below species-level distances indicated in bold.

K. rugosus Population	spiky (mcp)	spiky **(tep)**	elong. (msp)	elong. **(mcp)**	elong. (tep)	pouch. (ene)	elong. **(oma)**	pouch. **(anc)**	pouch. (pap)	elong. (amm)
spiky (mel)	0.062	0.067	0.090	0.092	0.089	0.081	0.090	0.085	0.077	0.075
spiky **(mcp)**		**0.008**	0.085	0.090	0.084	0.070	0.085	0.072	0.076	0.081
spiky (tep)			0.089	0.094	0.081	0.077	0.088	0.073	0.084	0.079
elong. **(msp)**				**0.004**	0.040	0.093	**0.003**	0.094	0.100	0.091
elong. **(mcp)**					0.045	0.097	**0.004**	0.099	0.102	0.090
elong. (tep)						0.086	0.040	0.087	0.090	0.100
pouch. **(ene)**							0.093	**0.004**	0.045	0.089
elong. (oma)								0.094	0.101	0.100
pouch. (anc)									0.047	0.083
pouch. (pap)										0.082
Outgroup (Rha)	0.122	0.134	0.151	0.154	0.138	0.125	0.150	0.100	0.129	0.362

Table 2: Pairwise uncorrected "p" distances for 1245bp of COI mtDNA among *K. waterhousei* populations found on *A. ammophila* (amm), *A. ancistrophylla* (anc), *A. cana* (can), *A. enervia* (ene), *A. loderi* (lod), *A. maranoensis* (mar), *A. melvillei* (mel), *A. microcephala* (mcp), *A. microsperma* (msp), *A. omalophylla* (oma), *A. papyrocarpa*, and *A. tephrina* (tep). Different *K. rugosus* gall structure abbreviations are given: spiky, elongate (elong.), and pouched (pouch.). *K. waterhousei* gall structures are uniform. Outgroup distances given by comparison with *Kladothrips* sister-genus *Rhopalothripoides* (Rha). Suspected below species-level distances indicated in bold.

K. waterhousei Population	(can)	(lod)	(amm)	**(oma)**	(mcp)	(mar)	(msp)	(ene)	(anc)
(pap)	0.101	0.058	0.088	0.083	0.090	0.082	0.100	0.022	0.034
(can)		0.091	0.071	0.071	0.072	0.103	0.017	0.095	0.095
(lod)			0.098	0.095	0.099	0.070	0.095	0.061	0.069
(amm)			0.047	**0.003**	0.096	0.067	0.080	0.096	-
(oma)					0.037	0.094	0.071	0.073	0.080
(mcp)						0.098	0.071	0.071	0.076
(mar)							0.099	0.073	0.085
(msp)								0.092	0.095
(ene)									0.035
Outgroup (Rha)	0.128	-	0.172	0.170	0.082	0.366	0.170	0.107	0.170

Table 3: Pairwise uncorrected "p" distances for 1245bp of COI mtDNA within *Kladothrips habrus*, *K. intermedius*, *K. waterhousei* species complexes. Host species abbreviations are as follows: *A. aneura* (ane); *A. cana* (can); *A. melvillei* (mel); A. *oswaldii* (osw); *A. papyrocarpa* (pap); and *A. pendula* (pen). Either or both host species or gall structure differentiates some populations. Suspected below species-level distances indicated in bold.

Population	*K. habrus* elongate (pen)	*K. intermedius* elongate (osw)	*K. waterhousei* (can)	*K. sterni* QLD (ane)
K. habrus elongate (mel)	0.063	**0.010**	0.080	0.124
K. intermedius pouched (osw)	0.076	0.050	0.096	0.134
K. waterhousei (pap)	0.084	0.046	0.094	0.117
K. sterni WA (ane)	0.144	0.119	0.140	**0.006**

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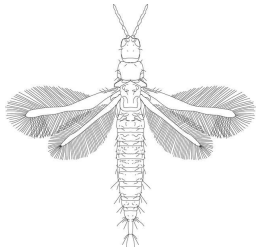


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