The gall-inducing habit has evolved multiple times among the eriococcid scale insects (Sternorrhyncha: Coccoidea: Eriococcidae)

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The habit of inducing plant galls has evolved multiple times among insects but most species diversity occurs in only a few groups, such as gall midges and gall wasps. This phylogenetic clustering may reflect adaptive radiations in insect groups in which the trait has evolved. Alternatively, multiple independent origins of galling may suggest a selective advantage to the habit. We use DNA sequence data to examine the origins of galling among the most speciose group of gall-inducing scale insects, the eriococcids. We determine that the galling habit has evolved multiple times, including four times in Australian taxa, suggesting that there has been a selective advantage to galling in Australia. Additionally, although most gall-inducing eriococcid species occur on Myrtaceae, we found that lineages feeding on Myrtaceae are no more likely to have evolved the galling habit than those feeding on other plant groups. However, most gall-inducing species-richness is clustered in only two clades (Apionomorpha and Lachnodius + Opisthoscelis), all of which occur exclusively on Eucalyptus s.s. The Eriococcidae and the large genus Eriococcus were determined to be non-monophyletic and each will require revision. © 2004 The Linnean Society of London, Biological Journal of the Linnean Society, 2004, 83, 441–452.


INTRODUCTION

The habit of inducing galls on plants has evolved multiple times among phytophagous insects, occurring in at least six orders. However, although there are more than 13 000 species of gall-inducing insects (Dreger-Jauffret & Shorthouse, 1992), most species diversity is clustered in only a few insect groups, such as the gall-midges (Diptera, Cecidomyiidae) (Dreger-Jauffret & Shorthouse, 1992) and the gall-wasps (Hymenoptera, Cynipidae) (Ronquist & Liljeblad, 2001). The phylogenetic clustering suggests that the taxonomic distribution of species diversity reflects radiations in those groups that have evolved the habit rather than many independent origins of the ability to induce plant galls.

Phylogenetic clustering of gall-inducing species is also apparent among the scale insects. Although gall inducers are known from ten coccoid families, the highest proportion (about 47%) of the more than 230 gallicolous species (Ferris, 1950; Beardsley, 1984; Gullan, 1984a; Ben-Dov, Miller & Gibson, 2003; Gullan, Miller & Cook, 2004) belongs to the Eriococcidae. This proportion is even greater (about 57%) if species that produce only simple pits or depressions are excluded, because almost all eriococcid gallers induce complex galls. The gall shapes are recognizably species-specific and structural complexity is often considerable, with the gall induced by a female sometimes resembling a fruit or other organ typically not found on that host plant. The shape of the gall appears to be independent of the host-plant species and the host-plant organ on which the gall grows and is determined by the species and sex of the scale insect (Gullan et al., 2004). Sexual dimorphism of gall morphology is apparent among most eriococcid species in which males are known to induce galls.

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The high number of gall-inducing eriococcids is not because of a numerical dominance by the Eriococcidae, which is only a distant fourth among the scale insect families in terms of total number of species (Ben-Dov et al., 2003). Therefore, another explanation is needed to explain the prevalence of gall-inducing taxa in this group.

Gall-inducing insects in general are prevalent in regions dominated by sclerophyllous vegetation (Price et al., 1998; Wright & Samways, 1998), leading to an argument that there is a selective advantage to galling in these habitats or intrinsic features of sclerophyll communities that may favour speciation among gallers (e.g. Mendonça, 2001). Although only 27% of eriococcid species worldwide occur in Australia, more than 80% of eriococcid gall-inducers occur in Australia (Miller & Gimpel, 2000; Gullan et al., 2004), predominantly on sclerophyllous plants of the Myrtaceae. If the gall-inducing eriococcid taxa belong to only one or a few lineages, then an extensive radiation in those groups may explain the prevalence of galling. However, if galling has evolved many times within Eriococcidae another explanation, such as common ecology, may be required.

Several recent studies have conflicted in their suggested relationships among Australian gall-inducing eriococcids. A molecular phylogeny of the scale insects (Cook, Gullan & Trueeman, 2002) suggests that galling has evolved at least twice among the eriococcids. Although some of the Australian eriococcid gallers formed a monophyletic group, the Casuarinae-feeding galling genus Cylindrococcus appeared to be more closely related to the non-galling type species of Eriococcus (E. buxi Boyer de Fonscolombe), and to Beesoniiidae and Stictococcidae. However, only one representative of each of the two latter taxa was included in the study and Apiomorpha, the most speciose gall-inducing scale insect genus, was not represented at all. Hodgson’s (2001) phylogenetic study of adult male morphology also places Beesoniiidae (which contains several gall-inducers) and Stictococcidae among the eriococcids but generic placements differ from those in Cook et al. (2002). Hodgson’s study suggested that the Australian gall-inducing genera Apiomorpha, Cystococcus, Lachnodius and Opisthoscelis were related to each other. However, Hodgson’s results should be treated with caution because male morphology may be subject to convergent selection in gall ing taxa.

In this study, we used DNA sequence data from three gene regions (two nuclear and one mitochondrial) to investigate whether the observed species richness of gall-inducing eriococcids in Australia may best be explained by:

1. an evolutionary radiation (possibly adaptive) within a single lineage, or
2. multiple origins of galling in separate lineages, suggesting that a common factor, such as a particular environmental condition, may have promoted the evolution of the gall-inducing lifestyle.

The study includes a broad taxonomic and geographical range of eriococcids, both gall ing and non-galling. The speciose genus Apiomorpha is represented for the first time in a molecular analysis of the Eriococcidae.

**METHODS**

**SPECIMENS AND DNA EXTRACTION**

Exemplars were chosen to sample the diversity of the Eriococcidae and to increase representation of groups whose placements in an earlier molecular study (Cook et al., 2002) were controversial. In particular, this study includes Eriococcus williamsii Danzig, the morphological sister taxon of E. buxi, and both described species of Cylindrococcus as well as additional representatives of Beesoniiidae and Stictococcidae. Pseudo-coccidae, previously identified as a suitable outgroup (Hodgson, 2001; Cook et al., 2002), was used to polarize trees. The taxa used, their current taxonomic classification, hosts, gall type (if applicable) and collection localities are given in Table 1. Voucher specimens of Australian taxa will be lodged in the Australian National Insect Collection (ANIC), CSIRO, Canberra, Australia.

Embryos and/or ovarian tissues were dissected from larger females. Small females and eggs were homogenized whole. DNA was extracted from fresh or ethanol-preserved specimens of embryos, eggs and/or ovarian tissues using the salting-out method of Sunnucks & Hales (1996). A single chloroform wash was performed prior to precipitation if solids or excessive pigments were present.

**PCR AND SEQUENCING**

The 5′ region of the small subunit ribosomal RNA gene (SSU rRNA) was amplified using the primers 2880 [for] (5′-CTGGTTTGATCTGCGCCAGTAG) (Tautz et al., 1988) and B-[rev] (5′-CCGCGGCTGCTGGCAGCAGA) (von Dohlen & Moran, 1995). Two new internal primers (E10for: GGAAGGAACGCTCTTATTAG, and E10rev: CGGTTTTGTATCTATAAGAGC) were designed for this region for Apiomorpha. An internal region of Elongation Factor 1-alpha (EF-1α) was amplified using primers M44-1 and rcm52.6 (Cho et al., 1995). These primers did not amplify EF-1α in all scale insects and new primers (EF-1αfor: GATGCTCCGGGACAYAGAG and EF-1αrev: GTTCTCCGACCCGAT) were designed internal to the original primers. An internal region of the mitochondrial COII gene was amplified using the
MULTIPLE ORIGINS OF GALLING IN ERIOCOCCIDS

Fisher Taq F1, Biotech) and 2 nuclear SSU rRNA (Van de Peer et al., 1997) at the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). The SSU rDNA data were aligned by eye with reference to the predicted secondary structure model for eukaryote nuclear SSU rRNA (Van de Peer et al., 1998). Some highly variable regions, particularly E10 helices of V2, did not have the same predicted secondary structures and were not able to be unambiguously aligned across all taxa. Alignable groups of sequences were offset with respect to other such groups. The introns of EF-1α also were not able to be aligned across taxa and were excluded from further analyses. Sequence data from each gene region were analysed separately because not all taxa could be amplified for COII or EF-1α. Additionally, the three data sets represent two genomes under different modes of inheritance.

Most methods of phylogeny reconstruction assume that the base composition is at equilibrium (stationary), with the base composition of homologous sequences the same for all taxa under study. Stationarity was tested using a chi-squares test as implemented in PAUP* 4.0b10 (Swofford, 2002) for each full data set and separately for third positions in COII and EF-1α. Substitutional saturation was assessed by plotting the transition–transversion ratio (ti/tv) against genetic distance and checking whether the curve had levelled.

A variety of different approaches to phylogenetic reconstruction were used, primarily to guard against undetected violations of underlying assumptions in any one model. It was assumed that, when different models and weighting schemes resulted in the same topology, the resultant phylogeny was robust in respect of inference method. Robustness does not imply that the phylogeny is accurate. Congruence among data sets provides increased confidence of relationships (Miyamoto & Fitch, 1995).

Bootstrap tests (Felsenstein, 1985) were conducted for maximum parsimony (MP) and distance-based analyses using 1000 replicates and the same settings as the original analyses. The inferred amino acid sequences were not used for phylogenetic analyses because the nucleotide compositional bias, and/or codon bias, may affect the inferred amino acid sequence (Foster, Jermin & Hickey, 1997; Singer & Hickey, 2000) and therefore exacerbate, rather than correct for, compositional bias.

PHYLOGENETIC ANALYSIS

Sequences were edited using Sequencher 3.02 (Gibbs & Cockerill, 1995) and the protein encoding regions (COII and EF-1α) were checked for the presence of stop codons in putative exons. A similarity search was performed on all sequences using the ‘basic blast’ option (Altschul et al., 1997) at the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). The SSU rDNA data were aligned by eye with reference to the predicted secondary structure model for eukaryote nuclear SSU rRNA (Van de Peer et al., 1998). Some highly variable regions, particularly E10 helices of V2, did not have the same predicted secondary structures and were not able to be unambiguously aligned across all taxa. Alignable groups of sequences were offset with respect to other such groups. The introns of EF-1α also were not able to be aligned across taxa and were excluded from further analyses. Sequence data from each gene region were analysed separately because not all taxa could be amplified for COII or EF-1α. Additionally, the three data sets represent two genomes under different modes of inheritance.

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BAYESIAN INFERENCE

Bayesian analyses were performed using MrBayes (Huelsenbeck & Ronquist, 2001) assuming a GTR+Γ model. Each run comprised four chains starting from random trees, and 500 000 generations with trees saved at each ten. Five runs were performed for each data set to check for convergence among runs. The majority rule consensus tree was calculated after the removal of trees saved during the ‘burnin’ period.

MAXIMUM PARSIMONY

Heuristic searches comprising 100 random addition sequence starting trees, TBR branch swapping and no maxtrees restrictions were performed using PAUP* 4.0b10 (Swofford, 2002). The SSU rDNA alignment was analysed under two weighting schemes: equal weights, and the V2 region weighted half that of the more conserved regions. Two weighting schemes for codon position (1st : 2nd : 3rd = 2 : 3 : 1 or 4 : 6 : 1) were used for the protein coding regions. Analyses were also run for COII with third codon positions excluded.

DISTANCE

The LogDet model (Lockhart et al., 1994) was used because base composition of third codon positions of
Table 1. Coccoidea species used in this study. Family classification of host genus is shown after genus name (Cr = Cornaceae, Cs = Casuarinaceae, Ct = Cactaceae, Cu = Cupressaceae, D = Dipterocarpaceae, E = Ertcaceae, Fb = Fabaceae, Fg = Fagaceae, I = Icacinaceae, L = Lamiaceae, M = Myrtaceae, Sn = Sapindaceae, St = Sapotaceae, W = Welwitschiaceae)

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<td><em>Opisthoscelis mammularis</em> Faggatt</td>
<td>Australia</td>
<td>Eucalyptus M</td>
<td>enclosing</td>
<td>AY795520</td>
<td>AY791954</td>
<td>AY795489</td>
</tr>
<tr>
<td><em>Ouroccocus</em> sp.</td>
<td>Australia</td>
<td>Eucalyptus M</td>
<td>N/A</td>
<td>AY795550</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Phacelococcus subcorticalis</em> Gullan &amp; Strong</td>
<td>Australia</td>
<td>Eucalyptus M</td>
<td>N/A</td>
<td>AY795494</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Scutare lanuginosa</em> Hoy</td>
<td>New Zealand</td>
<td>Pseudopanax</td>
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<td>AY795448</td>
<td>AY791961</td>
<td>AY795495</td>
</tr>
<tr>
<td><em>Sphaeroococcus</em> ferrugineus* Faggatt</td>
<td>Australia</td>
<td>Melaleuca M</td>
<td>rosette</td>
<td>AY795526</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Sphaeroococcus</em> pastulans* Green</td>
<td>Australia</td>
<td>Eucalyptus M</td>
<td>blister</td>
<td>AY795519</td>
<td>–</td>
<td>–</td>
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<tr>
<td><em>Sphaeroococcus</em> socialis* Maskell</td>
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<td>Melaleuca M</td>
<td>rosette</td>
<td>AY795527</td>
<td>AY791950</td>
<td>AY795487</td>
</tr>
</tbody>
</table>

† Introduced species, not native to collection locality.
both COII and EF-1α varied significantly among taxa (i.e. non-stationary). Characters were weighted by codon position (2 : 3 : 1) and a minimum evolution search criterion was used.

TRAIT MAPPING
The gall-inducing habit was mapped onto the SSU rRNA tree because it was generated using sequences from all taxa and was not strongly contradicted by either of the other two data sets. The putative origins of galling were assessed by MP optimization using ACCTRAN (accelerated transition) and DELTRAN (delayed transition) but other data, such as adult female morphology, also were considered.

Data on host use and gall-inducing habit (Tables 1 and 2) were obtained from Miller & Gimpel (2000), Gullan et al. (2004), ScaleNet (Ben-Dov et al., 2003) and our unpublished records to determine whether there may be a host-preference bias among Australian gall-inducing eriococcids. Three hierarchical categories of taxon assemblages were used: species, genera and lineages, with lineages being clades in which all taxa share the trait of interest. Host plants were assigned to one of two categories: Myrtaceae or ‘other family’. Myrtaceae was treated separately because it is the dominant host family in terms of eriococcid species richness. Myrtaceae was further divided into either Eucalyptus or ‘other genus’. Species of eriococcids were identified as being either galling or non-galling. Categories in which both galling and non-galling taxa occur were scored in counts for both. If one host-plant taxon is more favoured during the evolution of the gall-induction habit it should support more lineages of gall-inducers than do other host-plant taxa. However, if a host-plant taxon is favoured by eriococcids irrespective of whether or not they induce galls then this should be taken into account in assessing host-use bias.

RESULTS
There was no well-supported conflict among DNA sequence data sets (Figs 1, 2), although the resolution among some of the terminal taxa differed. For example, Apiomorpha was not recovered as a monophyletic group using EF-1α (Fig. 2A) despite strong support in the other two data sets. There was low support for most nodes in the COII data set (Fig. 2B), probably owing to substitutional saturation in all codon positions (>30% divergence). Similarly, third codon positions were saturated in EF-1α and there were only 25 potentially informative sites among first and second codon positions.

The gall-inducing eriococcids did not form a monophyletic group in any of the analyses. Three main clades of eriococcids, each comprising both gall-inducing and non-galling taxa, were recovered from all three data sets. The ‘BSE’ clade comprises representatives of three previously recognized families: Beeso- niidae, Stictococcidae and Eriococcus s.s. (Eriococcidae), thus rendering the Eriococcidae non-monophyletic. The ‘A’ (acanthococcid) clade contains a range of taxa including Dactylopius (Dactylopriidae), the gall-inducing Apiomorpha and Eriococcus aceris (Signoret), the type species of Acanthococcus if Eriococcus and Acanthococcus are recognized as separate genera (Melville, 1982). The ‘G’ (Gondwanan) clade comprises species from Australia and New Zealand and contains the subclade ‘MF’ (Myrtaceaefeeding) in

<table>
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<th>Host family</th>
<th>Myrtaceae</th>
<th>Other plant families</th>
<th>Total</th>
</tr>
</thead>
<tbody>
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<td>species</td>
<td>125 (82/43)</td>
<td>32 (3/29)</td>
<td>154 (85/69)</td>
</tr>
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<td>17 (11/8)</td>
<td>5 (2/3)</td>
<td>20 (13/9)</td>
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<td>lineages</td>
<td>11 (7/6)</td>
<td>6 (3/4)</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Host genus</th>
<th>Eucalyptus s.s.</th>
<th>Other genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>species</td>
<td>93 (66/27)</td>
<td>32 (16/16)</td>
</tr>
<tr>
<td>genera</td>
<td>12 (7/6)</td>
<td>6 (5/3)</td>
</tr>
<tr>
<td>lineages</td>
<td>6 (3/4)</td>
<td>6 (4/2)</td>
</tr>
</tbody>
</table>

Figure 1. Reconstruction of the evolution of the gall-inducing habit in the Eriococcidae on a phylogeny derived from SSU rDNA (18S) data using Bayesian analysis (GTR+Γ model). Bayesian posterior probabilities are shown above branches and maximum parsimony bootstraps are indicated below. Gall-inducing lineages are indicated by thick lines, with gall-type shown next to the taxon labels. Gall-type refers only to galls induced by females. An inferred loss of the gall-inducing habit is indicated by dashed lines. A schematic of adult female morphology is shown on the right of taxon labels. An inferred plesiomorphic eriococcid morphology is exemplified by Eriococcus and Madarococcus species; appendage loss and/or changes in body shape are associated with gall living.

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MULTIPLE ORIGINS OF GALLING IN ERIOCOCCIDS 447

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Gall type
- leaf fold
- pit
- rosette
- stem swelling
- enclosing

Ehrhonia cupressi
Dysmicoccus neobrevipes
Paracoccus sp.
Pseudococcus callitris
Pseudococcus sp.
Stictococcus sjostedti
Hockiana insolitus
Beesonia napiformis
Gallicoccus heckrothi
Eriococcus buxi
Eriococcus williamsi
Cylindrococcus spiniferus
Cylindrococcus casuarinæ
Callococcus acaciae
Callococcus pulchellus
"Sphaerococcus" pustulans
Opisthoscelis mammularis
Lachnodius sp.
Eremococcus turbinatus
Ascelis praemollis
Cystococcus echiniformis
Eriococcidae sp. "callo2"
"Sphaerococcus" ferrugineus
"Sphaerococcus" socialis
Madarococcus sp. "Nc"
Madarococcus viridulus
Eriococcus eucalypti sp.1
Eriococcus eucalypti sp.2
Eriococcus casuarinæ
Eriococcus fossils
Madarococcus totarae
Calycicoccus merwei
Eriococcus coccineus
Eriococcus sp. "CA1"
Dactylopius confusus
Dactylopius australis
Eriococcus acers
Eriococcus spurus
Eriococcus hakeae
Eriococcus coriaceus
Eriococcus tepperi
Eriococcus serratilobis
Eriococcus irregularis
Eriococcus leptospermi
Eriochiton spinosus
Scutare lanuginosa
Phacelococcus subcorticalis
Ourococcus sp.
Apiomorpha spinifer
Apiomorpha minor
Apiomorpha munita tereticornuta

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which all species feed exclusively on species of Myrtaceae. The BSE and the A clade each comprise taxa from the northern and southern hemispheres and four or more continents. The sole South African gall-inducing eriococcid, *Calycicoccus merwei* Brain, did not group strongly with any of the major eriococcid clades and its position varied among gene partitions.

Morphologically ‘typical’ eriococcids whose females have anal lobes and well-developed appendages, such as species found in the large genus *Eriococcus*, occur in each of the three main clades along with taxa with highly reduced or modified adult female morphology. A parsimony reconstruction of adult female morphology on the SSU rRNA tree (Fig. 1) infers that the morphology typified by *E. buxi* (the type species of *Eriococcus*) is plesiomorphic for the Eriococcidae.

Enclosing galls of complex morphology occur in three separate lineages (A, MF and *C. merwei*) (Fig. 1). Species that induce rosette galls also occur in multiple lineages (BSE and MF clades), as do those that induce only simple pits (G and MF clades).

The patterns of host use exhibit a strong phylogenetic component. The large monophyletic MF clade, comprising at least seven sampled genera, is restricted to species of Myrtaceae. The *Melaleuca*-feeding taxa ‘*Sphaerococcus*’ and Eriococcidae sp. (callo2) cluster together, separate from the eucalypt-feeding ‘*Sphaerococcus*’ *pustulans* Green. *Ascelis* and *Cystococcus*, the only two taxa on *Corymbia*, are sisters. However, the two speciose groups on *Eucalyptus*, *Apiomorpha* (A clade) and *Opisthoscelis* / *Lachnodius* (MF clade), are not closely related. Similarly, the gall-inducing genus *Cylindrococcus* is not closely related to other Australian eriococcids sampled from Casuarinaceae.

Myrtaceae is host to the majority of species of eriococcid in Australia (Table 2) and more than 65% of species on this host induce galls compared with only 10% of species feeding on other hosts inducing galls. Within Myrtaceae, a greater proportion of species feeding on *Eucalyptus* induce galls (>70%) than do so on other myrtaceous hosts.
Parsimony reconstructions using both ACCTRAN and DELTRAN suggest multiple origins of galling. There appears to be a minimum of seven origins of gall induction under DELTRAN, with one reversion to a non-galling habit in Callococcus. ACCTRAN infers five origins of galling with three reversions (BSE clade except Cylindrococcus; Callococcus; and the G clade minus MF) and regains of galling in Gallacoccus heckrothi Takagi and Madarococcus sp. nov. 'Nc'. Non-gall-inducing species of Callococcus are nested within the otherwise gall-inducing MF clade.

DISCUSSION

ORIGINS OF GALLING

The gall-inducing habit has probably arisen seven times among the taxa included in this study, as follows:

1. the Myrtaceae-feeding (MF) clade in Australia;
2. Cylindrococcus on Allocasuarina in Australia;
3. Calycicoccus merwei on Apodytes in Africa;
4. Apiomorpha (A clade) on Eucalyptus in Australia;
5. the leaf-folding Eriococcus sp. nov. 'CA1' (A clade) on Olneya in North America;
6. Madarococcus sp. nov. 'Nc' on Nothofagus in Australia;
7. Gallacoccus (Beesoniiidae) on Dipterocarpaceae in Asia.

Multiple origins of gall-induction, rather than multiple losses, are implicated because each of these groups has non-gall-inducing sister taxa with adult female morphology of the apparently plesiomorphic eriococcid type (anal lobes, well-developed legs and antennae). By contrast, each of the galling lineages has a different adult female morphology usually involving reduction of legs and antennae and modifications for plugging gall orifices. Adult females of pit-gall inducers exhibit few or no modifications relative to those that induce enclosing galls. Adult females within enclosed galls are not dorsoventrally flattened like the pit-gall formers and non-gall-inducers.

If the gall-inducing habit was ancestral for Australian eriococcids and had been lost multiple times then it would be expected that adult females of the sister taxa of gall-inducing groups would have morphologies reflecting their galling past. However, sister taxa of most of the gall-inducing taxa have well-developed legs and antennae typical of non-gall-inducing females of Eriococcidae and most other families. Exceptions, in which non-gall-inducing adult females are highly modified, occur in the BSE (Stictococcidae) and MF (Callococcus) clades. There are also several non-gall-inducing taxa in the A clade in which adult females have modified morphology but each of these have the habit of living in (Ourococcus) or under (Phacelococcus) bark.

Adult females of non-gall-inducing species of Callococcus, which is nested well within the MF clade, have no legs or anal lobes and the antennae are highly reduced; this is similar to the morphology observed in several galling genera of the MF group (e.g. Eremococcus and 'Sphaerococcus'). This is consistent with the non-gall-inducing Callococcus lineage having undergone morphological reductions during a period of gall-habitation and then subsequent return to a non-galling habit. If so, then the highly specialized lifestyle of gall-induction and associated loss of features associated with mobility has not led to an evolutionary dead end. Females of the two Callococcus species live on stems and produce waxy covers.

Male nymphs of five eriococcid genera (Eremococcus, Ascelis, Cystococcus, gall-inducing 'Sphaerococcus' and Eriococcidae sp. 'callo2') and several beesoniids (Mangalorea and Gallacoccus) develop within the chamber or bracts of the maternal gall (Gullan et al., 2004). Our results indicate that this habit has evolved independently in beesoniids and perhaps several times within the MF clade. However, our results do not unequivocally rule out the possibility that the above five MF clade genera may represent a monophyletic group. The five genera, although falling into two clades, have a Bayesian posterior probability of only 76 and no MP bootstrap support separating them on the SSU rRNA tree. Alternatively, the habit may have evolved in the ancestor of the MF group and subsequently been lost once in the remainder of the clade.

GALL MORPHOLOGY

Enclosing galls, pit galls and rosette galls have all apparently evolved more than once among the eriococcids. In general, these data do not suggest a transitional series from simple galls to more complex galls, as suggested for some other gall-inducing insects (e.g. Nyman, Widmer & Roininen, 2000). Inducers of simple galls (pits, leaf folds) are phylogenetically scattered and occur in three clades. They do not form a paraphyletic group within which complex gall-inducers are nested. However, a more comprehensive sampling across the eriococcids may help to determine whether there is an evolutionary transitional series of gall forms. In particular, the MF clade may provide the best opportunity for a phylogenetic study of gall evolution. This clade is well supported, speciose and comprises taxa that induce a diversity of gall forms yet all constituent taxa occur on hosts within a single plant family, thus minimizing the prospect of variability as a result of divergent host use.
HOST RELATIONSHIPS

About 80% of Australian eriococcid species occur on myrtaceous hosts despite this plant family comprising less than 10% of the species diversity of the Australian flora (Orchard, 1999). Our data suggest that even when lineages only, rather than species numbers, are considered there is an apparent bias towards Myrtaceae as a host. However, our data suggest that lineages feeding on Myrtaceae are no more likely to have evolved the gall-inducing habit than those on other plant groups (64% and 75%, respectively). It is apparent, though, that lineages that gall Myrtaceae are more species-rich than others. This relationship is primarily driven by the species richness of the gall-inducing eriococcids from two clades (Apionomorpha and MF clade) on Eucalyptus. It is unclear whether ‘S. pastulans, Lachnodius and Opisthoscelis represent independent shifts onto Eucalyptus or whether these three taxa are sisters with a common ancestor on Eucalyptus. Irrespective of the relationships among these three taxa, Apionomorpha and the MF clade may be considered to be independent observations because gall induction has evolved separately in each. Thus, eucalypt-feeding appears to have favoured higher species diversification among gall-inducing eriococcids.

It is probable that the high species richness of Eucalyptus and host-specificity has led to the high species richness of some eucalypt-galling eriococcid genera. For example, species of Apionomorpha (Eucalyptus-feeding) are more host-specific than are their non-gallicolous eucalypt-feeding relatives in the A clade (Gullan, 1984b; Miller & Gimpel, 2000; Cook, 2001). In addition, high species richness is associated with host specificity on eucalypts in other galler such as the Fergusosia/Fergusosina nematode/fly complex (Taylor, Davies & Giblin-Davis, 2003) and non-galling psyllids (Yen, 2002). A similar species radiation may have occurred among beesonoids on dipterocarps in Asia but most beesonid taxa are yet to be described (Takagi, 2001). Nevertheless, not all eriococcid taxa that gall eucalypts are species-rich (e.g. Floracoccus and Sphaerococcopsis; Gullan et al., 2004). Sister-group comparisons are required to test whether eucalypt-galling groups are indeed significantly more species-rich.

To date, there have been no tests of whether eucalypt-feeding gall-inducing insects have radiated in response to speciation of their hosts (cospeciation) or whether speciation has been associated with a recent radiation onto eucalypts. However, host use data (Cook, 2001) suggest that the co-speciation may have occurred within Apionomorpha because host specificity exhibits a strongly hierarchical pattern.

SYSTEMATICS AND BIOGEOGRAPHY

The three major clades of Eriococcidae recovered in this study correspond to the three previously identified by Cook et al. (2002). The recovery of the same three clades, despite increased taxon sampling in this study, and the occurrence of the three clades in three independent data sets suggest that the inferred relationships are robust. However, increased sampling from other scale insect families is still required to determine whether the concept of the Eriococcidae needs only to be expanded to include Beesoniidae, Stictococcidae and Dactylopiidae. If Dactylopiidae (represented only by the genus Dactylopis) belongs with the eriococcids, then there are serious nomenclatural implications because Dactylopiidae is an older name than Eriococcidae (Williams, 1969). If Eriococcidae is rendered further paraphyletic by the inclusion of major families such as Diaspididae and Asteroleticaniidae, then division of Eriococcidae should be considered.

Other taxonomic implications of this study are as follows. ‘Sphaerococcus’ ferrugineus Froggatt is not closely related to the Beesoniidae as suggested by Miller, Gullan & Williams (1998). Callococcus belongs with the eriococcids and not to the Asteroleticaniidae as currently placed (Miller et al., 1998). The large genus Eriococcus is not monophyletic, in agreement with the results of previous molecular data (Cook et al., 2002).

The Australian eriococcids are distributed across the three major eriococcid clades, two of which (G and A) also comprise taxa from New Zealand and two (BSE and A) have representatives from the Northern Hemisphere. A morphological study by Hodgson & Miller (2002) suggested close affiliations between some non-galling New Zealand and South American eriococcids. This worldwide distribution suggests that the current biogeographical patterns of some eriococcid clades may pre-date the break-up of Gondwana. A diverse eriococcid fauna was already present by the Late Cretaceous (Koteja, 2000), suggesting that eriococcids could have been widespread across Gondwana. However, many scale insects appear well suited for aerial dispersal as crawlers and not all current distributional patterns may represent vicariance. In particular, there is very little molecular divergence among several members within both the A and the BSE clades despite disjunct and widely separated distributions.

Sampling taxa from South America in particular will be important in determining systematic status and boundaries in Eriococcidae. In particular, several gall-inducing taxa are known from South America (Gullan et al., 2004) but their affiliations are not clear. Additionally, several of the South American taxa are
known from Myrtaceae and their relationship with the Australian fauna may help to elucidate further the association between eriococcids and Myrtaceae.

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