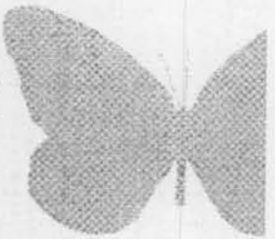
CHAPTER 18



Phylogenetic Relationships of the Riodinidae: Implications for the Evolution of Ant Association

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PHYLOGENETIC HYPOTHESES OF THE PAPILIONOIDEA

Although not for lack of study, the evolutionary history of the major lineages of "true" butterflies (Papilionoidea, including Hesperiidae) is still unknown, and multiple conflicting phylogenetic hypotheses exist in the literature. Assessing the systematic position of the metalmark butterflies (family Riodinidae) has been a particular challenge for this field. Furthermore, there is disagreement about the monophyly of this large group, which contains over 1,200 species. Most morphological studies place the riodinid butterflies as most closely related to the lycaenid butterflies, and identify the nymphalids as the closest relatives to this riodinid + lycaenid clade (Ehrlich and Ehrlich 1967; Kristensen 1976; Scott and Wright 1990; de Jong et al. 1996a) (fig. 18.1A, B). These relationships have been inferred with a variety of phylogenetic methods and are supported by a number of adult, larval, and pupal synapomorphies, although few are universal or uniquely derived. An alternative hypothesis of the placement of the riodinids was proposed by Robbins (1988a). Based on a cladistic analysis of nine character states among four characters of the foreleg coxa, trochanter, and basal femur, Robbins suggested that the Riodinidae are more closely related to the Nymphalidae than to the Lycaenidae (fig. 18.1C), and split the lycaenids into two groups, which may not compose a monophyletic lineage: Lycaeninae-Theclinae-Polyommatinae and Lipteninae-Poritinae-Miletinae-Curetinae.

Two molecular studies have explored papilionid relationships, but their results conflict with each other and with the morphological hypotheses (fig. 18.1D, E). In comparing nucleotide characters from the 28s subunit of nuclear ribosomal RNA, Martin and Pashley (1992) found no close

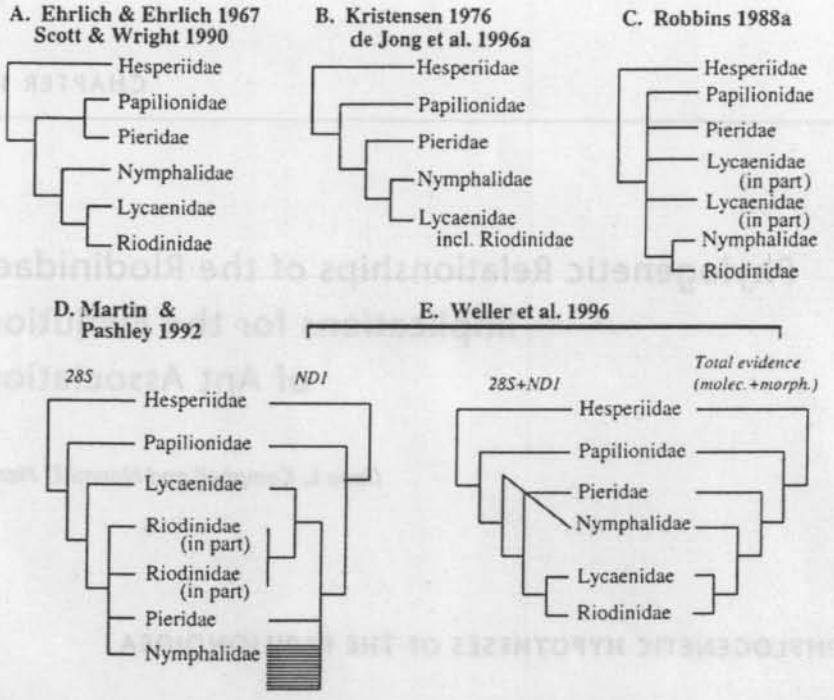

Figure 18.1 Results of previous studies addressing the relationship of the Riodinidae to the other butterfly families.

phylogenetic relationship between the Riodinidae and the Lycaenidae, and instead found the monophyly of the Riodinidae uncertain. Analysis of a portion of a second gene, the mitochondrial NADH dehydrogenase subunit 1 (ND1) (Weller et al. 1996), resulted in a monophyletic interpretation of the Lycaenidae + Riodinidae, but did not resolve the sister to the Lycaenidae + Riodinidae clade, nor did it recover a monophyletic Nymphalidae. When characters from both genes were analyzed in combination, the riodinid + lycaenid relationship was recovered, and the Pieridae came out as the sister to the riodinid + lycaenid clade. A total evidence analysis combining the molecular data from both genes with morphological characters taken from all five of the morphological studies mentioned above once more supported a riodinid + lycaenid clade with a sister relationship to the Nymphalidae (Weller et al. 1996).

TAXONOMIC RANKING

Previous studies of butterfly relationships vary in their interpretation of the taxonomic rank of the riodinids, with some conferring familial (Eliot 1973; Harvey 1987; Robbins 1988a; Martin and Pashley 1992; Weller et al. 1996) and some subfamilial (Ehrlich 1958; Kristensen 1976; Scott and Wright 1990;

de Jong et al. 1996a) status. In this chapter we refer to the Riodinidae as a family due to the precedence of this terminology in studies examining within-riodinid relationships (Stichel 1928; Clench 1955; Harvey 1987; Robbins 1988a). Hence, we use the term Lycaenidae to refer to the non-riodinid lycaenids *sensu* Eliot (Eliot 1973; but not Eliot in Corbet et al. 1992).

ANT ASSOCIATION IN THE PAPILIONOIDEA

The Riodinidae and the Lycaenidae are distinct among the Papilionoidea in that they have evolved the ability to form complex larval associations with ants (myrmecophily: Hinton 1951; Pierce 1987; DeVries 1991; Fiedler 1991; Pierce et al. 2002). In many cases, these are mutualistic associations whereby the larvae secrete nutritious solutions from specialized glands (ant organs) to multiple species of ants in exchange for protection from predators and parasites (Malicky 1970; Pierce and Easteal 1986; DeVries 1991; Axen and Pierce 1997). Some myrmecophilous larvae have evolved separate organs that are thought to secrete chemicals to appease the ants and further mediate larval-ant interactions (Clark and Dickson 1956; Malicky 1970; Claassens and Dickson 1977; DeVries 1988b, 1991). While many consider ant association to have evolved once in a riodinid + lycaenid ancestor and been subsequently lost in multiple lineages (Hinton 1951; Vane-Wright 1978; Scott 1984; Scott and Wright 1990), it has also been argued that riodinid and lycaenid ant organs may not be homologous, since they are found on different larval segments in these two families. Thus, instead of having a single origin in the Papilionoidea, ant association may have evolved independently in the riodinids and in the lycaenids (DeVries 1991, 1997; see also Fiedler 1991).

In order to settle the placement of the riodinid butterflies and to examine the evolution of myrmecophily from a phylogenetic perspective, molecular sequence characters from the 3′ exon of *wingless* were generated (Campbell et al. 2000). This developmentally active nuclear gene evolves rapidly in nymphalid butterflies (at rates exceeding those of the mitochondrial genes *Cytochrome Oxidase I* and *Cytochrome Oxidase II*) and has been informative for reconstructing relationships in a large dataset of nymphalid butterflies (Brower and DeSalle 1998). Applications of sequence characters derived from *wingless* to problems involving relationships within riodinids and among butterfly families have confirmed the utility of *wingless* for problems at this phylogenetic level (Campbell 1998; Campbell et al. 2000). In this chapter we review the phylogenetic relationships among riodinid, lycaenid, and nymphalid butterflies as recovered by *wingless*, and we examine the implications of the phylogenetic placement of the Riodinidae with respect to the other butterfly families for our understanding of ant association in the Papilionoidea.

METHODS

Sampling Scheme, Outgroups, and Available Voucher Specimens

Taxa were selected to represent each of the main lycaenid, riodinid, and nymphalid lineages (table 18.1). Two representatives of the Pieridae and one species of the Papilionidae were also included. A hesperiid representative was included as an outgroup based on previous studies of butterfly systematics, which agree on the Hesperiidae as the basal lineage of the Papilionoidea. Adult butterflies were collected as fresh specimens and the bodies stored in 100% ethanol at −80°C. Wings were retained as voucher specimens in the Harvard Museum of Comparative Zoology (riodinids, lycaenids, *Papilio glaucus*, and *Ancyloxypha numita*) and the American Museum of Natural History (nymphalids and *Pieris rapae*). PCR and sequencing were carried out as described by Campbell et al. (2000). All sequences have been submitted to Genbank (for accession numbers, see Campbell et al. 2000).

Phylogenetic Analysis

Our extensive exploration of the signal in *wingless* characters, and our phylogenetic analysis of *wingless* characters using various character weighting strategies as well as model-based analytical methods for these taxa, are discussed in detail elsewhere (Campbell 1998; Campbell et al. 2000). Here we present a conservative parsimony analysis in which third codon position transitions are excluded on the basis that these characters were found to be saturated in some taxa (however, they do contain signal for other taxa, particularly taxa in the family Riodinidae; see Campbell et al. 2000). Heuristic parsimony searches were performed with TBR branch swapping and fifty random addition replicates using the computer program PAUP*4 test versions d49 and d56, kindly provided by D. Swofford (Swofford 1998b). One hundred bootstrapping replicates were done to assess nodal support.

PHYLOGENETIC PATTERNS

Parsimony analysis of *wingless* characters excluding third position transitions recovered four most parsimonious trees. A strict consensus of these trees is shown in figure 18.2. The results of this analysis are summarized by family below.

Table 18.1 Taxa used in this study and their classification

Family	Subfamily	Tribe	Species	Locality
Riodinidae	Euselasiinae		*Euselasia* sp.	Ecuador: Sucumbios Province
	Hamearinae		*Taxila haquinus*	Malaysia: FRIM Kepong
			Abisara saturata	Malaysia: Kuala Woh, Papah
	Riodininae	incertae sedis	*Crenina actoris*	Ecuador: Sucumbios Province
		Eurybiini	*Eurybia* sp.	Ecuador: Sucumbios Province
		Mesosemiini	*Mesosemia* sp.	Ecuador: Sucumbios Province
		Riodinini	*Riodina lysippus*	Ecuador: Sucumbios Province
		Charitini	*Sarota* sp.	Ecuador: Sucumbios Province
		Emesini	*Emesis* sp.	Ecuador: Sucumbios Province
		Nymphidiini	*Nymphidium cachrus*	Ecuador: Sucumbios Province
		Helicopini	*Helicopis cupido*	Ecuador: Sucumbios Province
		Lemoniini	*Thisbe irena*	Ecuador: Sucumbios Province
Lycaenidae	Poritiinae	Poritiini	*Poritia phama*	Malaysia: Genting Tea Estate
			Simiskina pheretia	Malaysia: Awana FR, Pahang
		Liptenini	*Baliochila minima*	Kenya: Arabuko-Sokoke
	Curetinae		*Curetis bulis*	Malaysia: FRIM Kepong
	Miletinae	Miletini	*Miletis ancon*	Malaysia: FRIM Kepong
		Liphyrini	*Liphyra brassolis*	Australia: Queensland
			Spalgis epius	Malaysia: Genting Tea Estate
	Theclinae		*Habrodais ganus*	USA: Nevada, Lang Crossing
			Jalmenus daemeli	Australia: Queensland, Townsville
	Polyommatinae		*Candalides geminus*	Australia: Queensland, Burra Range
			Jamides alecto	Malaysia: FRIM Kepong
	Lycaeninae		*Heliophorus kiana*	Malaysia: Kinabolu Park
Nymphalidae	Heliconiinae		*Heliconius erato*	French Guiana: Pointe Macouria
	Libytheinae		*Libytheana carineta*	Brazil: Rondonia, Ariquemes
	Satyrinae		*Cercyonis pegala*	USA: New York, Ithaca
	Morphinae		*Morpho helenor*	Brazil: Rondonia, Ariquemes
	Limenitidinae		*Limenitis arthemis*	USA: New York, Caroline
			Diaethria clymena	Brazil: Rondonia, Ariquemes
	Nymphalinae		*Hypolimnas misippus*	Reared in captivity, ex. C. Clarke
			Siproeta steneles	Brazil: Rondonia, Ariquemes
	Danainae		*Danaus plexippus*	Colombia: Meta, Villavisencia
	Ithomiinae		*Melinaea maenius*	Brazil: Rondonia, Ariquemes
Pieridae	Pierinae		*Pieris rapae*	USA: New York, Ithaca
			Delias sp.	Australia: NSW, Lismore
Papilionidae	Papilioninae		*Papilio glaucus*	USA: Colorado, Gunnison
Hesperiidae			*Ancyloxypha numita*	USA: Massachusetts, Boston

Note: Classifications are according to Harvey 1987 (riodinids), Eliot 1973 (lycaenids), and Harvey 1991 (nymphalids).

Among-Family Relationships

Strong bootstrap values support riodinid monophyly, lycaenid monophyly, and a sister relationship between the riodinids and the lycaenids (see fig. 18.2). The power of *wingless* is considerably lower, however, at the next deeper level of comparison. It is unable to establish unambiguously the sister taxon to the riodinid + lycaenid lineage. This analysis recovers the Nymphalidae + Pieridae + Papilionidae as the sister clade to the Riodinidae + Lycaenidae; however, this arrangement is not supported by bootstrapping, and should be interpreted very cautiously.

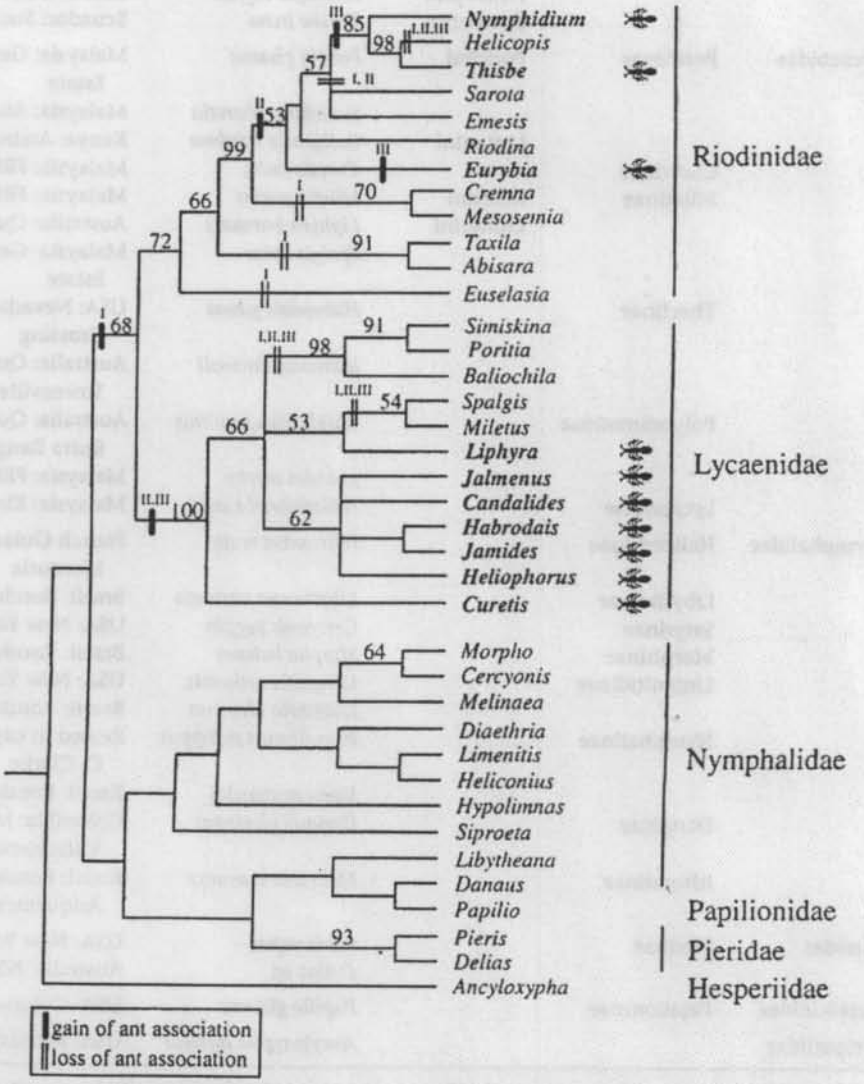

Figure 18.2 Strict consensus of the four most parsimonious trees resulting from an analysis in which third position transitions were excluded. Bootstrap values are shown for nodes with 50% or greater support. Taxa that have ant organs are shown in boldfaced type and identified with an ant icon.

Within-Family Relationships

Riodinidae

The *wingless* data establish strong evidence for the monophyly of the Riodinidae, countering the possibility of a polyphyletic Riodinidae raised by Martin and Pashley (1992). Molecular studies have yet to test the phylogenetic affiliation of the rare monotypic riodinid subfamilies Styginae and Corrachiinae, although recent morphological analyses place these within the Riodinidae (Harvey 1987; Robbins 1988a, 1988b; Scott and Wright 1990). Most relationships within the Riodinidae are well resolved and well supported by bootstrapping (see fig. 18.2) and are largely concordant with the most recent hypothesis of riodinid subfamilial and tribal relationships (Harvey 1987).

Except for the position of *Euselasia, wingless* recovers the same riodinid topology under different analytical conditions, indicating that these are robust conclusions (maximum likelihood, distance methods, and parsimony under different weighting strategies were examined; see Campbell et al. 2000). In some analyses, *Euselasia* was found to be the sister group to the Hamearinae, represented by *Abisara* + *Taxila*. The ant-associated tribes Lemoniini, Nymphidiini, and Eurybiini form two clades, with the Eurybiini evolving earlier and the Lemoniini and Nymphidiini derived from a common ancestor. A larger study using more taxa (Campbell 1998) and a morphological analysis (Penz and DeVries 1999) indicate that although the Lemoniini and Nymphidiini make up a monophyletic group, they are not, as Harvey suggested, distinct lineages. Relationships within the riodinids, in an analysis involving more extensive taxon sampling, are discussed further in Campbell (1998).

Lycaenidae

Very few morphological characters supporting the monophyly of the Lycaenidae exist in the systematic literature, although only two studies have suggested that the lycaenids are paraphyletic (Scott 1984; Robbins 1988a, 1988b). Both of these studies consider the lycaenid subfamily Curetinae to be more closely related to the riodinids than to the lycaenids, although Scott later changed this view to a monophyletic Lycaenidae (Scott and Wright 1990). The *wingless* gene contributes multiple characters that strongly support a monophyletic Lycaenidae including the Curetinae.

Our *wingless* analysis places *Curetis* as the most basal lycaenid group; this interpretation is supported by Eliot (in Corbet et al. 1992). The curetines share many morphological characters with the Riodinidae, especially characters that are not found in other lycaenids, such as a large anepisternum,

uncus, and transtilla and tibial spurs (Scott and Wright 1990). The basal position of the curetines is consistent with the parsimonious interpretation that these are symplesiomorphic traits that were lost once, in the ancestor of the non-curetine lycaenids. Noise or conflicting signal generated by third position transitions in *wingless* reduces support for this basal relationship, however, and analyses employing alternative weighting schemes (for example, when all third positions are included in the parsimony analysis: Campbell et al. 2000) place the Curetinae as a highly derived lycaenid lineage (as do Scott and Wright 1990), albeit with no bootstrap support. The position of the curetines requires further examination. Reconstruction of lycaenid relationships is currently in progress using *wingless* and other molecular characters as well as more extensive taxon sampling.

Nymphalidae

In the analysis presented here, the Nymphalidae are found to be paraphyletic with respect to the pierid representatives and *Papilio*. However, none of the recovered relationships among the Nymphalidae have bootstrap support greater than 50%, and when different weighting schemes or analytical methods are used, the topology of the nymphalids is very different (Campbell et al. 2000). For example, unweighted parsimony finds the Nymphalidae to be polyphyletic with respect to the riodinid + lycaenid clade (again, with little or no bootstrap support: Campbell et al. 2000).

Monophyly of the Nymphalidae is not supported by *wingless*, yet at the same time *wingless* does not strongly refute the possibility of nymphalid monophyly (i.e., nodes contradicting nymphalid monophyly are not supported by bootstrapping; furthermore, forcing the constraint of monophyly of all nymphalids or forcing monophyly of all nymphalids except *Libytheana* in heuristic searches does not significantly increase tree length). Short internal branch lengths render almost all within-nymphalid relationships unstable and highly dependent on the weighting method used for analysis. Furthermore, the informative changes among nymphalids appear to consist of mostly third position changes, and only a few first and second position changes. In riodinids and lycaenids, on the other hand, first and second position informative sites are much more common (Campbell 1998). This may explain the difference in the utility of the *wingless* gene in recovering relationships within and between these families. The monophyly of the Nymphalidae and its relationship to the Lycaenidae + Riodinidae is still in need of rigorous investigation using other genes and more extensive taxonomic sampling.

It is notable that, using characters from a portion of the ND1 gene, Weller et al. (1996) also did not recover the monophyly of, or any resolution within the nymphalids. On the other hand, characters from the 28s gene

recovered the Nymphalidae, but not riodinid + lycaenid monophyly (Martin and Pashley 1992). These findings suggest that the inability to resolve all families at once may not be due to the shortcomings of a particular gene itself, but might instead be due to biological differences in the radiations of these lineages, or a different rate of molecular evolution in the Nymphalidae. Alternatively, there is some evidence that *wingless* may be evolving at a different rate in the riodinids and lycaenids than in the nymphalids (Campbell 1998). Differing rates of evolution among taxonomic groups may reflect interesting evolutionary histories of the organisms or of the genes; this difference among the riodinids, lycaenids, and nymphalids is being examined further (D. Campbell, P. J. DeVries, and N. Pierce, unpub.). For these reasons, substitution rates (and thus phylogenetic signal) for a particular gene in one taxonomic group may not translate to other groups, even closely related ones, of the same taxonomic ranking. This complicates the process of choosing a gene for phylogenetic reconstruction based on the results of studies performed on other similarly aged radiations of taxa.

IMPLICATIONS FOR THE EVOLUTION OF ANT ASSOCIATION

As reviewed in the previous section of this chapter, phylogenetic analysis of *wingless* indicates that the Lycaenidae and Riodinidae, both of which have evolved specialized larval adaptations for mediating associations with ants, belong to a single clade. Although other Lepidoptera are known to engage in interactions of various kinds with ants (Hinton 1951), no cases of myrmecophily of a similar nature are known in other butterflies. It is also clear from this work that the evolutionary pattern of ant association is not a simple one. For instance, when species possessing ant organs are mapped onto the phylogeny in figure 18.2, we find that the most basal riodinid lineages do not have any ant-associated members. This finding implies that myrmecophily has been lost and/or gained multiple times.

The phylogenetic approach is powerful in that it allows us to access this interesting pattern, and it becomes the basis for framing the question of how ant association evolved. Knowing the relationship between the riodinids and lycaenids enables us to form three hypotheses: (1) ant association is a shared, derived character that evolved at the base of the riodinid + lycaenid lineage and was apomorphically lost in non-myrmecophilous branches in both families; (2) the ancestor to the riodinids and lycaenids was not associated with ants, and ant relationships evolved independently in the riodinid and lycaenid lineages; (3) ant association evolved independently in the lycaenids and in the two myrmecophilous riodinid lineages: Eurybiini and Lemoniini+ Nymphidiini (DeVries 1991, 1997). Yet even a thorough phylogenetic knowledge of these groups will not, by itself, distinguish among these hypotheses.

Table 18.2 Inferred numbers of evolutionary events for three hypotheses for the evolution of ant association when myrmecophily is mapped onto the phylogeny in figure 18.2

	Gains	Losses	Total
Hypothesis I	1	7–9[a]	8–10[a]
Hypothesis II	2	4–6[a]	6–8[a]
Hypothesis III	3	3	6

[a] Number of losses is dependent on the resolution of *Sarota, Emesis,* and *Riodina* (Riodinidae).

Although we can theoretically estimate the minimal number of evolutionary changes required by each hypothesis (table 18.2), interpreting the most parsimonious pattern of gains and losses of myrmecophily is an arbitrary distinction when the mechanism underlying these gains and losses is not understood. That is, we do not know, for example, whether one gain and two losses is more or less likely than two gains and six losses. Thus, examining the origin of myrmecophily in the butterflies requires more than a phylogenetic analysis.

The homology of the structures that riodinid and lycaenid caterpillars use in their myrmecophilous lifestyles (ant organs) provides perspective on ant association. If, for instance, lycaenid and riodinid ant organs are not homologous, then it is likely that ant association has also evolved independently. DeVries (1991, 1997) has begun to explore this question. He notes that the riodinid larvae secrete food for ants through a paired set of "tentacle nectary organs" (TNOs), located on the eighth abdominal segment (A8). The lycaenids, on the other hand, use a nontentacular organ (the dorsal nectary organ, or DNO) on the seventh abdominal segment (A7) for this purpose (fig. 18.3). Likewise, the riodinids secrete chemical stimuli to ants through paired anterior tentacle organs (ATOs) on the third thoracic segment (T3), whereas the lycaenid organs with equivalent function (tentacle organs, or TOs) are located on the eighth abdominal segment (A8). Thus the organs for feeding ants and the organs for controlling ants chemically are located on different body segments in lycaenids and riodinids, and since they do not derive from the same body part, DeVries interprets them as having independent origins. Under this interpretation, a myrmecophilous ancestor of the riodinids and lycaenids would be very unlikely.

However, despite their different placements, histological studies of riodinid and lycaenid ant organs show unmistakable parallels, especially the three kinds of tentacular organs (ATOs, TNOs, and TOs). In both lycaenids and riodinids, these organs are glandular and secretory (Ross 1964, 1966; Kitching 1983; Kitching and Luke 1985; DeVries 1988b), have glandular tissue that is connected via ducts to terminal setae (Ross 1964; Malicky 1970; Cottrell 1984; DeVries 1988b), and are retracted into the body when

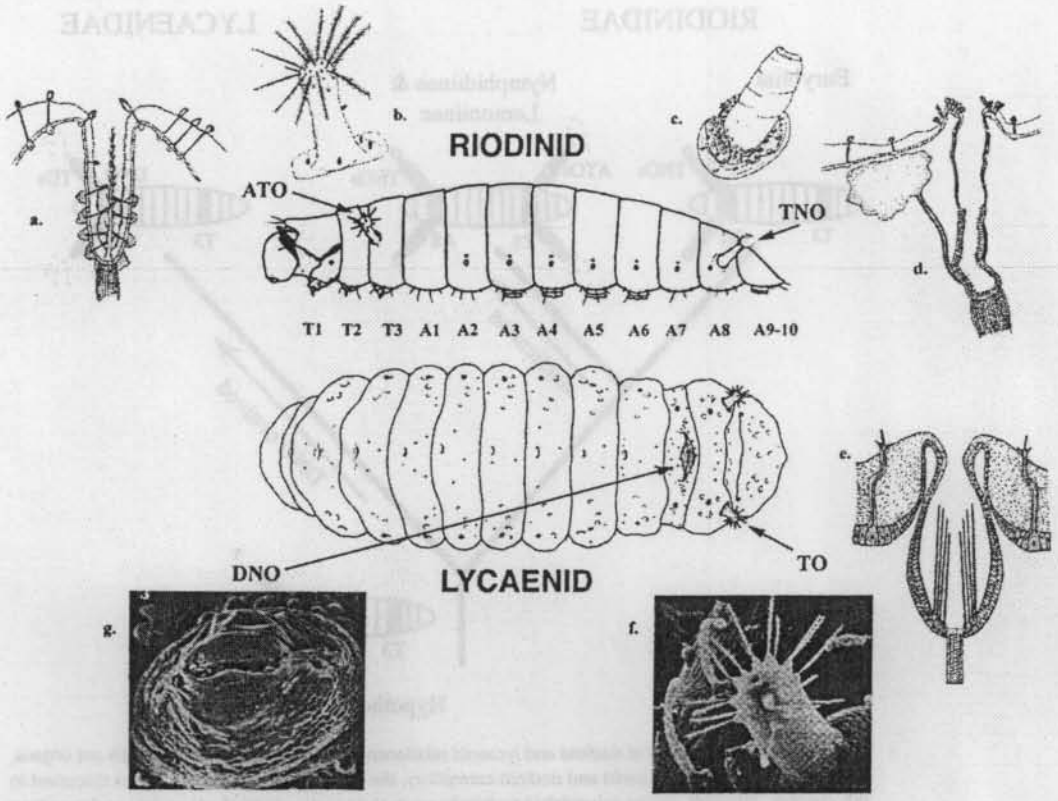

Figure 18.3 Placement of riodinid and lycaenid ant organs. Electron micrographs and histological diagrams demonstrate the similarity of structure among these organs. Body segments are labeled T1–T3 (thoracic segments 1–3) and A1–A10 (abdominal segments 1–10). (A) Histological section of a riodinid anterior tentacle organ (ATO; *Anatole rossi*). (B) Line drawing of a riodinid ATO (*Anatole rossi*). (C) Line drawing of a riodinid tentacle nectary organ (TNO; *Anatole rossi*). (D) Histological cross section of a riodinid TNO (*Anatole rossi*). (E) Histological cross section of a lycaenid tentacle organ (TO; *Phaedrotes piasus*). (F) Electron micrograph of a lycaenid TO (*Lysandra coridon*). (G) Electron micrograph of a lycaenid dorsal nectary organ (DNO; *Lysandra coridon*). (Riodinid line drawing from De Vries 1988b; A–D from Ross 1964; E from Hinton 1951; lycaenid line drawing and F–G from Kitching and Luke 1985.)

not in use. Clark and Dickson's (1956), Claassens and Dickson's (1977), and Hinton's (1951) illustrations of the histology of lycaenid tentacular organs show a structure similar to that in Ross's (1964) illustration of the riodinid counterpart (fig. 18.3).

The tentacular organs might be more parsimoniously explained as homologous structures, with shifts in function and position over time. The required changes in function (between secreting food and secreting chemical substances) may be trivial if food secretions also contain chemicals that are important in mediating interactions with ants, as Pierce (1983, 1989) and DeVries (1997) have suggested. Thus the nectar-supplying TNOs on the eighth abdominal segment of riodinids would be homologous to the chemical-secreting organs on the lycaenid eighth abdominal segment (Cottrell 1984; Kitching and Luke 1985). Furthermore, the riodinid

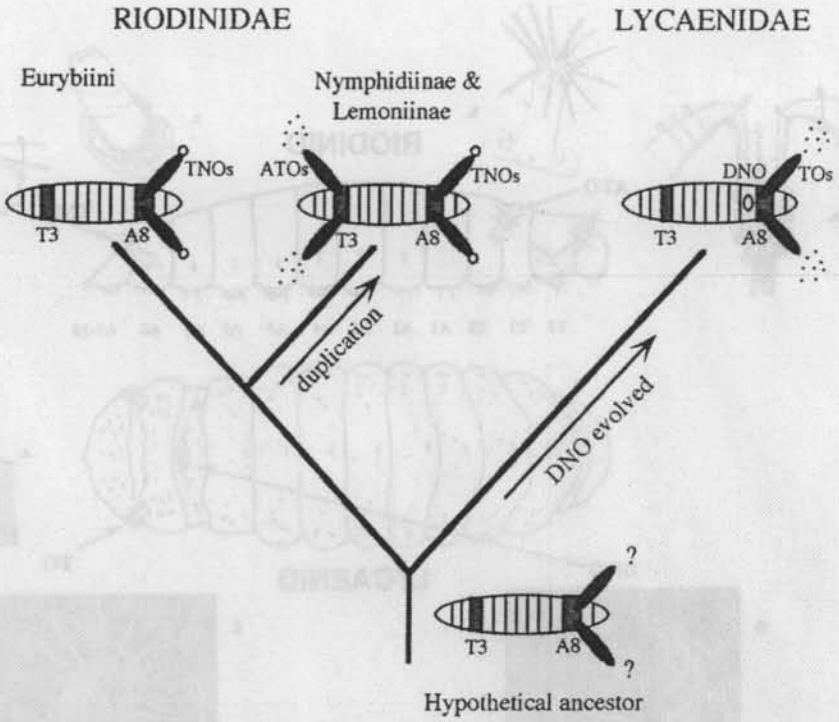

Figure 18.4 Simplified tree of riodinid and lycaenid relationships, showing only lineages with ant organs. The diagrams show, for lycaenid and riodinid caterpillars, the full complement of ant organs discussed in this chapter, although species (especially lycaenids) vary in their endowment of ant organs and may have a subset of this complement. DNO = dorsal nectary organ. Tentacle organs (TNO, ATO, TO) are shown in black, and their function is indicated next to the diagram by a series of dots (volatile chemical secretions) or a droplet (liquid food secretion). ? represents the unknown secretion of the ancestor.

chemical-secreting ATOs on the third thoracic segment might be explained as an iterative homologue to the tentacular organs on the eighth abdominal segment under a broader definition of homology; that is, as structures derived from the same ancestral developmental cascade (Wagner 1994; Lauder 1994; Abouheif 1997). This kind of evolutionary change is especially possible in metameric organisms in which much positional identity is under the control of homeobox genes, allowing regulatory changes to cause duplications or shifts among segments (Roth 1991; Abouheif 1997).

A possible model of the evolution of ant organs, based on phylogenetic relationships and this concept of serial homology, is illustrated in figure 18.4. In this model, the riodinid + lycaenid ancestor is postulated as having one set of tentacular organs on the eighth abdominal segment. These organs diversified in function, becoming food producers in riodinids and chemical secretors in lycaenids. At some point in lycaenid evolution, the DNO developed on segment A7 (perhaps as an independent organ, or perhaps as a serially repeated pair of tentacular organs, subsequently modified into a single structure) to provide food rewards for ants. In the riodinids, one

lineage (the Eurybiini) retained ancestor-like organs, while in the more derived Nymphidiini + Lemoniini lineage the tentacular organs were serially duplicated on the third thoracic segment and their function changed to chemical secretion. While speculative, this hypothesis provides a mechanism for the existence of genetically homologous ant organs on different segments, and relieves the requirement for entirely separate evolutionary events to independently create ant organs de novo. This level of homology allows the possibility that ancestral riodinids and lycaenids had ant organs (and were myrmecophilous) even if the ant organs on extant riodinids are not homologous on the basis of their position (*sensu* DeVries 1991, 1997) to those on extant lycaenids.

It is too early to determine whether ant association, tentacular organs, or the genetic pathways specifying tentacular organs in the lycaenid + riodinid lineage are ancestral or have evolved independently. Phylogenetic reconstruction will eventually resolve the relationships within the lycaenid and riodinid families, and will be crucial in reconstructing the evolution of variation within ant-associated lineages. However, interpreting the origins of myrmecophily also requires testing the theories based on phylogenetic understanding through an exploration of the developmental genetics, physiology, and structure of the ant organs themselves.

One possible starting point for future research in this direction is to compare expression patterns in riodinid and lycaenid larvae for genes involved in the development of ant organs. Homologous structures have been examined in this way in other systems; for example, in studies of the genes involved in the establishment of eyespots on butterfly wings in different taxa (see Brakefield and Monteiro, chap. 12 in this volume). While comparable expression patterns in these ant organs would not in themselves guarantee functional homology of a gene in different taxa, they would suggest that the development of the organs may be parallel, and that a regulatory shift producing iterative structures is a possible evolutionary scenario. The genetics of ant organ development is unknown, but investigations of related systems have furnished potentially involved genes. For example, a promising starting candidate is the gene *distal-less* (*dll*), which shows expression at the tip of most protruding structures, such as limbs, antennae, and setae, in a variety of Lepidoptera, as well as in other insects and invertebrates (Carroll et al. 1994; Panganiban et al. 1995). Different expression patterns of *dll* certainly would indicate different origins of the riodinid and lycaenid organs. Similar *dll* expression patterns might be a result of homology among ant organs, or could represent convergence, and would require testing of other gene products, working down the developmental cascade if possible. Research in insect developmental genetics is now quickly making the job of finding candidate genes more and more feasible. A more immediate challenge is finding representative ant organ–bearing riodinid species whose biology and larval host

plants are well enough known that larvae can be reared in numbers in the laboratory. This needs to be accomplished soon, before habitat destruction reduces the number of species from which to choose.

SUMMARY

Phylogenetic characters derived from the *wingless* gene provide the first strong molecular evidence for two important phylogenetic conclusions: (1) that the riodinids and lycaenids each form monophyletic groupings, and (2) that the riodinids and lycaenids are sister lineages. Interestingly, while *wingless* provides robust support for relationships within and between the riodinids and lycaenids, it is less informative about nymphalid relationships. Nymphalids appear to cluster as part of an unsupported group along with pierid and papilionid representatives; *wingless* does not consistently recover a monophyletic Nymphalidae, nor does it resolve relationships within the Nymphalidae with confidence. Elucidating riodinid origins enhances our understanding of how ant association may have evolved in the Papilionoidea. We present a model of the evolution of ant association based on the phylogenetic relationships recovered here coupled with a genetic interpretation of ant organ homology and development, which suggests that ant association may have been an ancestral feature of riodinid and lycaenid butterflies. Comparing expression patterns of genes involved in ant organ development is suggested as a possible direction for testing this hypothesis.

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