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Aphytophagy in the Miletinae (Lycaenidae): Phylogeny, Ecology, and Conservation

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**APHYTOPHAGY IN THE MILETINAE (LYCAENIDAE):
PHYLOGENY, ECOLOGY, AND CONSERVATION**

by

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Old Dominion University in Partial Fulfillment of the
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ABSTRACT

APHYTOPHAGY IN THE MILETINAE (LYCAENIDAE): PHYLOGENY, ECOLOGY AND CONSERVATION

John Mathew

Old Dominion University, 2003

Co-Directors of Advisory Committee: Dr. Deborah A. Waller

Dr. Naomi E. Pierce

Less than 1% of all Lepidoptera are aphytophagous; of these, a considerable proportion is found in the family Lycaenidae. The aphytophagous Lycaenidae are believed to have arisen from a mutualistic template involving ant attendance. With this association firmly in place, it is a relatively simple shift to exploitation, either of the ants themselves, through active carnivory on the brood/trophallactic feeding from adults, or by carnivory on ant-tended homopterans, with little to no interference by the ants. Among lycaenids, aphytophagy has arisen several times; most spectacularly in the subfamily Miletinae, where all of the approximately 150 species are presumed or known to be aphytophagous. With the exception of the North American species *Feniseca tarquinius*, the subfamily is restricted to the Old World, in particular, Africa and South-East Asia. The focus of this study was a comprehensive review of aphytophagy in the Miletinae, viewed in light of phylogenetic and ecological patterns. A representative genus, *Thestor*, endemic to southern Africa, was chosen for intense phylogenetic study, where the relationships of nearly all 29 morphological species and subspecies were analyzed, employing the molecular genes Cytochrome Oxidase 1 (CO1) and subunit 5 of nicotinamide dinucleotide (ND5). The resultant phylogenies generated were used to inform life-history characters where known. The enigmatic Nearctic species, *Feniseca tarquinius*, was studied for life-history traits in Massachusetts, New Hampshire, Maine and New Brunswick, with particular emphasis on diet and vibrational signaling. It was shown that the species produces the longest known acoustic pulse train of any lycaenid. SEM examination of final instar larvae was also undertaken for purposes of comparison with a close relative, the Palearctic species *Taraka hamada*. An aphytophagous habit adds an extra dimension of complexity to a feeding habit, involving both the prey item

and its host-plant resource, rendering its practitioners immediately susceptible to extinction pressure. Conservation concerns for the Lycaenidae, with special emphasis on aphytophagous forms, were hence considered, from the perspective of IUCN Red Data listing, and suggested causes for decline, and appropriate conservation measures discussed, and applied to specific case studies

Dedicated to my family, for accompanying me the length of the way;
Deborah Waller, for permitting me to go,
and
Naomi Pierce, for allowing me to stay.

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As the inspired author, Vikram Seth, was once wont to say, "My debts are manifold and various," and I take his words as my own in framing this heartfelt statement of gratitude. Seeing as the debt transcends borders both political and oceanic, I place my sentiments in order of temporal priority, beginning in India in the year 1995, continuing through Norfolk, Virginia (though prefixed with a detour to other parts of the United States), and concluding 8 years thence in Cambridge, Massachusetts.

India

My first acknowledgment of thanks must be laid at the feet of my parents, the geneticist Dr. Chona Mathew and the once historian and indefatigable advocate of the creative arts Mrs. Elizabeth Mathew, who made my path to academic achievement possible. To the first I attribute my love of biological process (the tantalizing possibilities of dihybrid test crosses) apart from an ingrained passion for cricket and chess, and to the latter, theatre, music, writing, literature, and spirituality, which have in such large measure, become a way of life. Their lasting support, even when they have not always found it easy to understand the choices their son has made, leaves me more grateful than I can say. Thank you.

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To Kerala, Tamil Nadu, India, and South Asia, I reaffirm my fealty. I have drawn upon these my native and adopted regions so much during my years in the United States for my sense of identity, and in turn I hope that I have been, in some wise, an ambassador, for them and for the developing world as a whole. They are the subjects of my visions, now and for the future, and it is my privilege to belong to them.

The United States

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SECTION 1

INTRODUCTION

Aphytophagy, or feeding on non-plant sources, is the preferred food habit among most insect orders with only 9 of 30 orders typically associated with plant feeding (Kristensen 1991). However, less than 1% of all Lepidoptera are aphytophagous (Strong et al. 1984; Common 1990; Pierce 1995). A significant proportion of predatory/parasitic Lepidoptera belongs to the family Lycaenidae, and in particular, members of the subfamily Miletinae (Pierce 1995). In the Lycaenidae, aphytophagy is speculated to have been derived from mutualistic associations with ants (Pierce 1995; Rand et al. 2000; Pierce et al. 2002) that characterize many representative species in the family. Such divergence from mutualism appears to take one of two forms: a) the lycaenid caterpillar ceases to produce reward secretions while chemically fooling the ant into continuing to tend b) the lycaenid throws off the trappings of appeasement myrmecophily and turns entirely aphytophagous.

Let us briefly examine each of these options.

a) Cessation of or minimized secretion: It is possible that larvae in mutualistic associations receive the services of an ant guard while returning little in exchange – the larvae might simply mimic the more abundant and valuable resources that plant nectaries and their homopteran analogues (aphids, coccids and membracids) afford ants by way of nitrogen-rich secretions (Pierce & Mead 1981). Since the tending and guarding of nectaries is already an integral part of the behavioral repertory of many ant species, it might be a comparatively simple matter for lycaenids in a mutualistic association to have evolved opportunistic bribery and perhaps even outright deception.

b) Aphytophagy: Feeding on sources of food that are not derived from plants *sensu stricto* (feeding on plant sap will be considered aphytophagous in this definition) is a remarkably minuscule part of dietary diversity in lepidopterans (0.0012% - figure calculated from Pierce 1995), as per known life histories. Of these,

The journal model is taken from *Conservation Biology*.

approximately 30% belong to the Papilionoidea, which effectively means the Lycanidae (including the Riodininae), aphytophagy not having been demonstrated in the other papilionoid families. The life histories of only two riodinines that are aphytophagous have been described in the literature (De Vries et al. 1992), leaving the overwhelming remainder in the Lycaenidae proper. It is generally considered that aphytophagy is a derived condition in the Lepidoptera and indeed, phylogenetic analyses (Rand et al. 2000; Pierce et al. 2002) indicate that aphytophagy is likely to be particularly tippy in most clades, with the exception of the lichen feeding Liptinini, the diversely aphytophagous Miletinae, and the genera *Lepidochrysops*, *Maculinea*, *Niphanda* and *Acrodipsas* in an otherwise overwhelmingly phytophagous subfamily Lycaeninae. With some exceptions, a general feature of the aphytophagous lycaenids is the possession of pore cupolae organs that ostensibly provide appeasement secretions to the ants, or at the very least, prevent the ants from attacking the caterpillars themselves. Once tolerance is achieved evolutionarily, it would seem to be a relatively simple dietary shift to feed upon the homopteran attendees of the ants directly, or to fool the ants chemically or behaviorally into allowing them access into their nest-chambers, where the caterpillars could either feed upon the ant-brood directly, or by trophallaxis (Dodd 1902; Cottrell 1984; Maschwitz et al. 1985; Maschwitz et al 1988; Johnson & Valentine 1986; Fiedler 1991; Fiedler 1993; Dejean 1991; Dejean & Beugnon 1996).

It is against this backdrop that I have divided the sections of my dissertation into a hierarchical order of study as follows:

Section 2 is a treatment of the subfamily Miletinae from the perspective of described life histories, biogeography, and systematics, both morphological and molecular. Questions concerning the placement of particular tribes in the subfamily are discussed, as the origins of aphytophagy in the subfamily itself, the biogeographic radiation of included genera and what this may inform us about their dietary patterns, whether these are more phylogenetically or ecologically constrained, if at all. This discussion stems from an overview of mutualistic associations between lycaenid caterpillars and their attendant ants, and the features of such mutualisms that may potentially have given rise to the origins of aphytophagy in the group.

Section 3 examines the phylogenetic relationships of the 29 morphologically described species of the southern African miletine endemic, the genus *Thestor*. Unlike the Miletinae as a whole, previously existing phylogenetic trees based on morphological considerations are not readily available, and so informal species groupings, as suggested by the work of Alan Heath and Ernest Pringle (in preparation) have been tested against molecular phylogenies estimated from characters derived from sequences of the mitochondrial genes Cytochrome Oxidase I (COI), and subunit 5 of Nicotinamide Dinucleotide 5 (ND5). The superficial distinction of grouping species into yellow *Thestor* and black *Thestor* has been tested by enforcing monophyly upon these groups and comparing it with the original tree, and significant differences determined employing the Kishino Hasegawa, Templeton and winning sites tests. A partition heterogeneity test is also applied to a combined tree including both COI and ND5. Finally, life history, morphology, and distribution characteristics are mapped on to the most parsimonious tree.

Section 4 addresses life-history aspects of the only Nearctic miletine, *Feniseca tarquinius*. In particular, larval morphology based on SEM studies, acoustic signatures, diet and ant attendance are examined. All of these factors, based on original observations in the field and laboratory, will be compared with life history information accrued from the literature. The conservation implications for this admittedly ephemeral species will also be discussed.

Section 5 reviews the current conservation status of lycaenid butterflies, with special reference to aphytophagous species, and describes selected case studies.

Section 6 summarizes Chapters 2, 3, 4 and 5 and concludes the dissertation by tying together overarching themes.

SECTION 2

THE MILETINAE: A COMPREHENSIVE OVERVIEW

The working hypothesis for the higher classification of the Lycaenidae is based upon morphological characteristics and derived from Eliot (1973; revised in Corbet et al. 1992). The Lycaenidae will be treated here as including five subfamilies - the Curetinae, the Miletinae, the Poritiinae, the Riodininae and the Lycaeninae. The Riodininae is cautiously included in this treatment, recognizing that irrespective of its ultimate position (i.e. distinct family, or subfamily under the Lycaenidae), it forms a monophyletic clade and a sister clade to the rest of the Lycaenidae as a whole (Ehrlich 1958; Campbell et al. 2000). By far, the largest subfamily is the Lycaeninae, including approximately 3,700 species (Bridges 1988), followed by the Riodininae, with over 1,200 species (Harvey 1987; Campbell et al. 2000) the Poritiinae with 572 species, the Miletinae with 140 species, and the Curetinae, with approximately 20 species (Bridges 1988) (Figure 2.1).

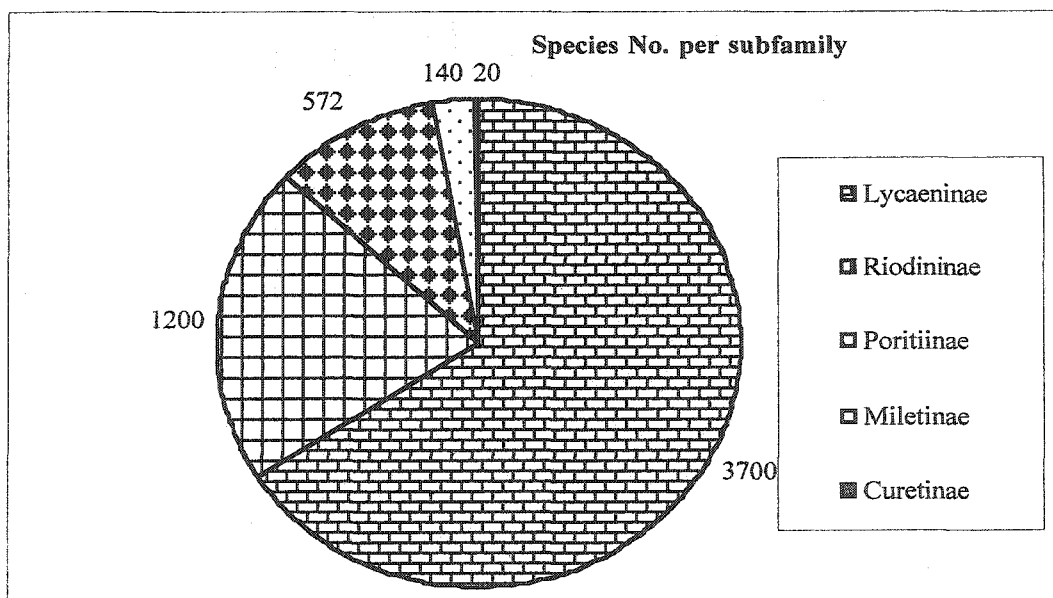


Figure 2.1. *Lycaenid Species Numbers* (Harvey 1997; Bridges 1988; and Campbell et al. 2000).

Phylogenetic placement of lycaenid subfamilies based upon previous studies

The most recent delineation of subgroups for the butterflies as a whole (Hedyloidea, Hesperoidea, and Papilionoidea) is by Ackery et al. (1999) where they relate an identification key based on morphological characters to the results of their preliminary phylogenetic analysis (de Jong et al. 1996). Eliot (1973) divided the Lycaenidae, excluding the Riodininae and Stygidae (the latter inclusive of only one species, the enigmatic South American *Styx infernalis*), into eight subfamilies. Kristensen's (1976) inclusion of the then Riodinidae to within the Lycaenidae resulted in the recognition of ten subfamilies by Ackery and Vane-Wright (1984), as originally classified by Ehrlich (1958). Eliot (1990) reduced the number to five – the Riodininae, the Curetinae, the Miletinae, the Poritiinae and the Lycaeninae, based on an intuitive evaluation of 19 selected characters scored across 33 tribes. This simplified classification suggests that all five subfamilies are monophyletic, however, de Jong et al. (1996) suggest that only the Riodininae emerge consistently as a monophyletic group, apart from the Curetinae, a family that comprises only a single genus. Ackery et al. (1999) suggest that a possible relationship between the Poritiinae, Miletinae and Curetinae could be supported by the shared character of the forewing coxa arched upward distally and sometimes extending beyond its articulation with the trochanter (Robbins 1988). However, their phylogenetic analysis does not support this grouping. Eliot (1973) and Harvey (1987) state that the pterothoracic tibial spurs are absent in both the Poritiinae and the Miletinae, supporting a relationship between them based on this loss apomorphy. This loss, however, also recurs in two of six species of *Eumaeus* (Robbins personal communication cited in Ackery et al. 1999). The parsimony analyses of de Jong et al. (1996) suggest that the Poritiinae and Miletinae cluster as a paraphyletic assemblage in relation to the subfamily Riodininae + the genus *Liptena*. Robbins (1988) shows that the sister taxon of the Miletinae is unresolved, being either Poritiinae or Curetinae. Eliot (1973; in Corbet et al. 1992) is of the view that the Curetinae formed a monophyletic unit with the Poritiinae and Miletinae. Scott and Wright (1990) place the Poritiinae as a sister taxon to the Miletinae. By contrast, Campbell et al. (2002) place the Curetinae as sister to the Lycaeninae, with the

Militianae basal to the resultant clade, and the Poritiinae basal to the Miletinae. Some of these hypotheses are depicted in Figure 2.2.

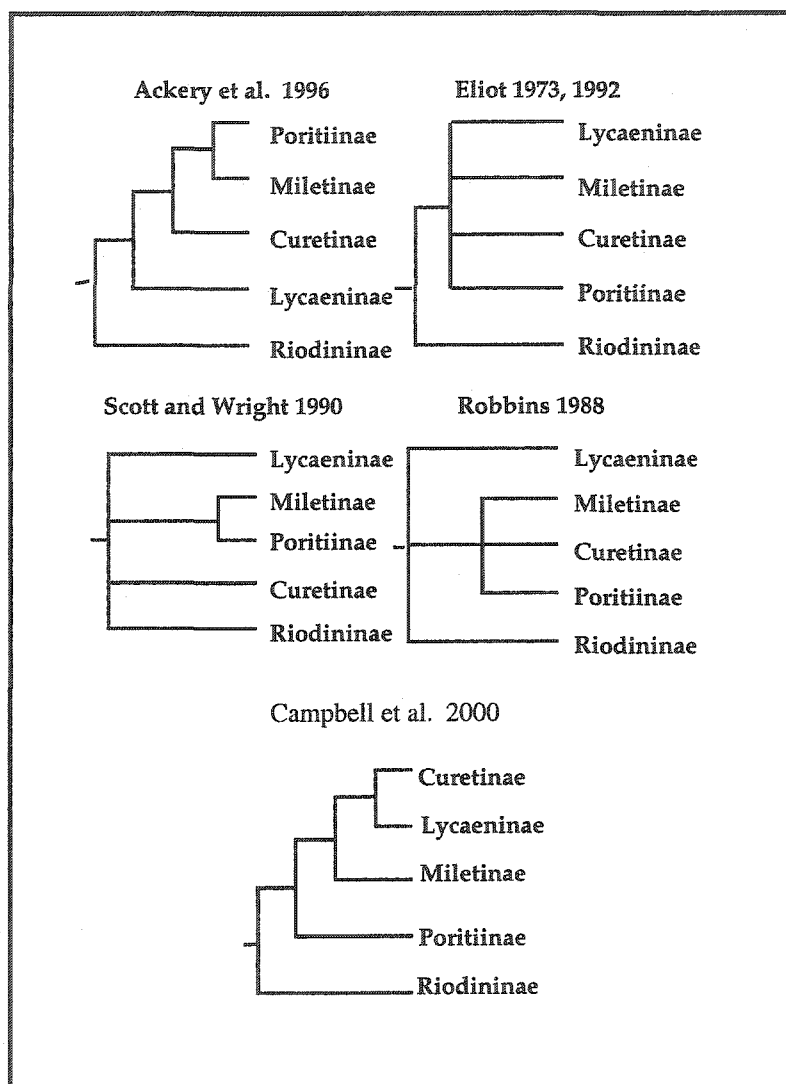


Figure 2.2. Hypotheses regarding subfamilial relationships among the Lycaenidae.

Adaptations to myrmecophily in the Lycaenidae

Life histories for about 20% of an estimated 6000 species in the Lycaenidae have been described, and of these, about 75% associate with ants, i.e. they are myrmecophilous (Pierce et al. 2002). Ant association is also found among the riordinines, though probably not to the same extent as in the lycaenids (De Vries 1990). However, since these two

groups account for about 30% of some 17,280 species of butterflies estimated to occur worldwide (Shields 1989), larval association with ants is clearly a significant component of butterfly ecology (Baylis & Pierce 1992). Although lycaenid-ant associations can be parasitic, commensal, or mutualistic (Hinton 1951; Atsatt 1981; Cottrell 1984; Pierce 1987), mutualisms in which the fitness of each partner is increased by the action of the other appear to be by far the most common type of interaction. The larvae of many species have specialized glands that visibly secrete droplets of food, which are consumed by ants. The association is primarily mediated through several specialized exocrine glands that secrete substances that appease ants, and in many cases, reward them. Three types of such organs are well known. These include the pore cupolae organs (PCOs), the dorsal nectary organ (DNO) and the tentacular organs (TOs).

The PCOs are small glandular structures that are derived from hairs, with the hair shaft transformed into a sieve-plate with numerous muted pores of 0.1-0.2 μm diameter. PCOs occur almost ubiquitously in both lycaenid larvae and pupae (Malicky 1969; Ballmer & Pratt 1988), with the possible exception of the moth butterfly, *Liphyra brassolis* (Fiedler 1991). This brood predator lives inside the nests of the aggressive weaver ant, *Oecophylla smaragdina*, where an unusually thickened cuticle of around 50 μm serves as an alternative defensive strategy against ant attack (Malicky 1970). Morphology and distribution of the PCOs, however, differ markedly between the subgroups of the Lycaenidae, Miletinae and Curetinae (De Vries et al. 1986; Kitching 1987; Fiedler 1991). Hinton (1951) suspected that PCOs were the source of attractive substances to ants, a fact established by Malicky (1969; 1970), who showed that PCOs cause intensive antennation behavior in ants attending lycaenid larvae. The chemical signals released by the PCOs appear non-specific in facultatively myrmecophilous lycaenids, because ants from different genera or even subfamilies frequently react similarly to the same lycaenid immatures (Ballmer & Pratt 1988; Fiedler 1991). The PCOs may hence represent a key innovation that allowed ancestral lycaenids to benefit from enemy-free space in the presence of ants (Atsatt 1981; Pierce et al. 2002) with ant appeasement ultimately giving rise to more sophisticated mutualisms (De Vries 1991).

The DNO is an epidermal gland located on the dorsum of the seventh abdominal segment. Usually the opening of the DNO is surrounded by a cluster of PCOs and very

often by a field of specialized setae (Clark & Dickson 1956; Malicky 1969; Downey & Allyn 1978; Kitching & Luke 1985; Fiedler 1988). The DNO secretes droplets of a clear fluid when stimulated by ants via antennation, which are eagerly imbibed by the ants (Malicky 1969). In contrast to the PCOs, a functional DNO is usually not present in the pupal stage (though see Downey 1965). The DNO is not as ubiquitous as the PCOs; it is only known from the subfamily Lycaeninae, where it is secondarily missing in a number of species and genera. Kitching and Luke (1985) coined the term 'myrmecoxenous' to include all those species lacking a DNO. In the Miletinae, Kitching (1987) mentions a structure on the seventh abdominal segment of *Allotinus major* that he calls 'pseudo-Newcomer's organ.' However, the glandular nature of this organ has not been proved.

The TOs are a pair of eversible epidermal tubes located on the dorsum of the eighth abdominal segment of many lycaenid caterpillars. The TOs are everted upon stimulation by ants, when the caterpillars crawl about or are disturbed. Thomann (1901) supposed the TOs to be scent organs influencing the ants' behavior. Malicky (1969; 1970), however, could not detect any glandular structures within the TOs (though see Pierce & Nash 1999 for photographic descriptions of glandular TOs for *Jalmenus evagoras*), nor any reaction of the ants towards the eversion of these organs. He concluded that TOs of lycaenid caterpillars were rudiments of formerly important organs. This view has been challenged by reports that attendant ants do respond to the eversion of the TOs with alertness or even alarm behavior (Elfferich 1963; 1965; Downey & Allyn 1979; Fielder & Maschwitz 1988; 1989). This reaction is usually observed in a radius of a few mm around the TOs. Furthermore, not all ant species react to the TOs of a given lycaenid species. The overwhelming majority that does tends to belong to the subfamily Formicinae (Fiedler 1991). Exceptions including *Aricia morronensis*, where the attendant dolichoderine ant *Tapinoma* shows characteristic excited runs (Munguira & Martin 1988), and *Iridomyrmex anceps* (Dolichoderinae) responds similarly to *Jalmenus evagoras* (Pierce personal communication). Axen and Pierce (1998), have shown that as the number of tending ants increases, solitary larvae increase the rate of secretion from the dorsal nectary organ, but decrease the eversion of the tentacular organs. For larval groups of three or more, the secretion rate is almost three times lower than for solitary larvae; tentacular displays also decrease with increasing group size. Group size also affects ant attendance, with per

capita attendance decreasing with increased group size (Axen & Pierce 1998). TOs are present in the larvae of the subfamilies Curetinae and Lycaeninae. With the interesting exception of the genus *Aslauga*, larvae of the Miletinae do not possess TOs (Cottrell 1984). TOs are often missing in species that have the DNO, and species with TOs often lack DNOs (Fiedler 1991).

Apart from the exocrine glands involved in mutualisms, there are passive protective features that pre-adapt most lycaenid caterpillars to myrmecophily. The most important of these is an unusually thick and tough cuticle, (e.g. larvae of *Liphyra brassolis*), which can be 5-20 times thicker than for other lepidopteran larvae of comparable size (Malicky 1969). Additionally, many lycaenid larvae are onisciform, i.e., the dorsum is weakly rounded, while the flat venter adheres tightly to the substrate (Malicky 1969; Cottrell 1984). Finally, most lycaenid larvae can retract their heads completely under their prothoracic shields. Thus, the most vulnerable organs obtain protection against possible ant attacks, and the shape of their body together with the toughness of their cuticle allow most larvae to withstand occasional hostile reactions.

Synapomorphies in the Miletinae

Within the Miletinae, *Liphyra* and *Miletus* are not grouped together in the parsimony analyses of de Jong et al. (1996). There are no unambiguous synapomorphies that unite them and their close relatives as a well-founded subfamily (de Jong et al. 1996). The Miletini in the sense of Ackery et al. (1999) is broad, ostensibly based on the classification of Scott and Wright (1990), subsuming the tribes Spalgini, Lachnocnemini and Miletini. The fact that all miletines are aphytophagous, living on homopterans, homopteran secretions, ant regurgitations, or ant brood (Cottrell 1984) does not make for a synapomorphy for the subfamily, since similar aphytophagous behavior is seen in other subfamilies of the Lycaenidae. The results of the exhaustive phylogenetic analysis conducted by Pierce et al. (unpublished) for the Lycaenidae as a whole will throw more light on the placement of the Miletinae within it.

Tribal and generic considerations in the Miletinae

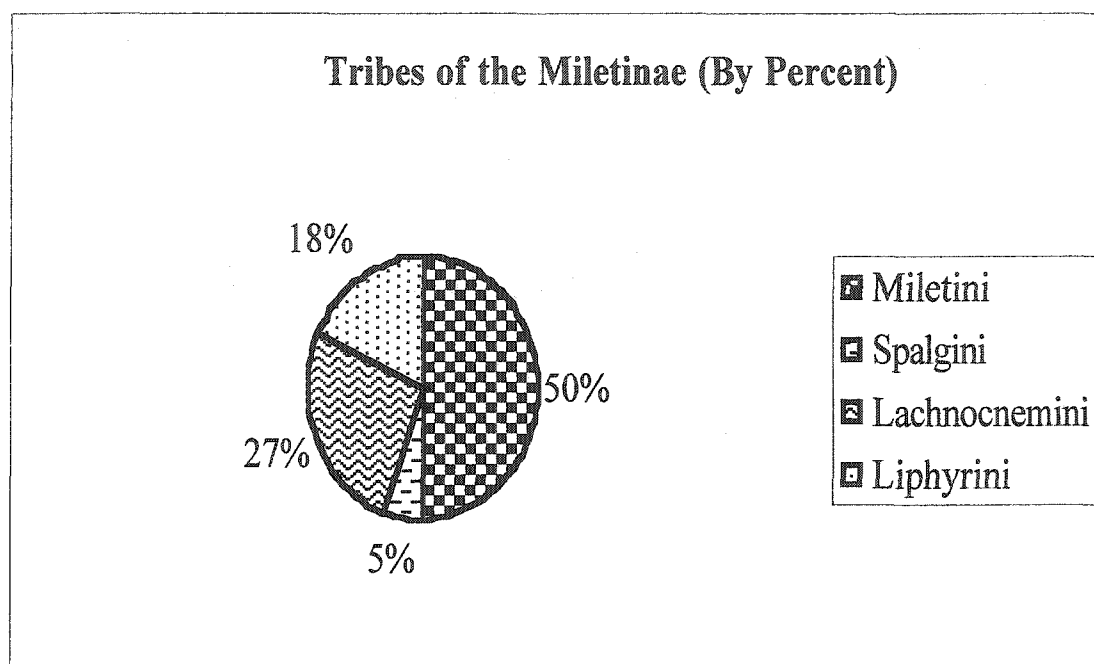


Figure 2.3. Representative miletine tribes, by percent.

Table 2.1. Breakdown of the Miletinae by lower taxa and biogeography.

| <i>Tribe</i> | <i>Genus</i> | <i>Number of Species</i> | <i>Biogeography</i> |
|---------------|---------------------|--------------------------|-----------------------|
| Miletini | <i>Allotinus</i> | 33 | Oriental |
| | <i>Logania</i> | 11 | Oriental |
| | <i>Lontalius</i> | 1 | Oriental |
| | <i>Megalopalpus</i> | 4 | Afrotropical |
| | <i>Miletus</i> | 26 | Oriental |
| Spalgini | <i>Feniseca</i> | 1 | Nearctic |
| | <i>Spalgis</i> | 5 | Oriental-Afrotropical |
| | <i>Taraka</i> | 2 | Oriental-Palaeartic |
| Lachnocnemini | <i>Lachnocnema</i> | 13-14 | Afrotropical |
| | <i>Thestor</i> | 28 | Afrotropical |
| Liphyrini | <i>Aslauga</i> | 22 | Afrotropical |
| | <i>Euliphyra</i> | 3 | Afrotropical |
| | <i>Liphyra</i> | 2 | Australian-Oriental |

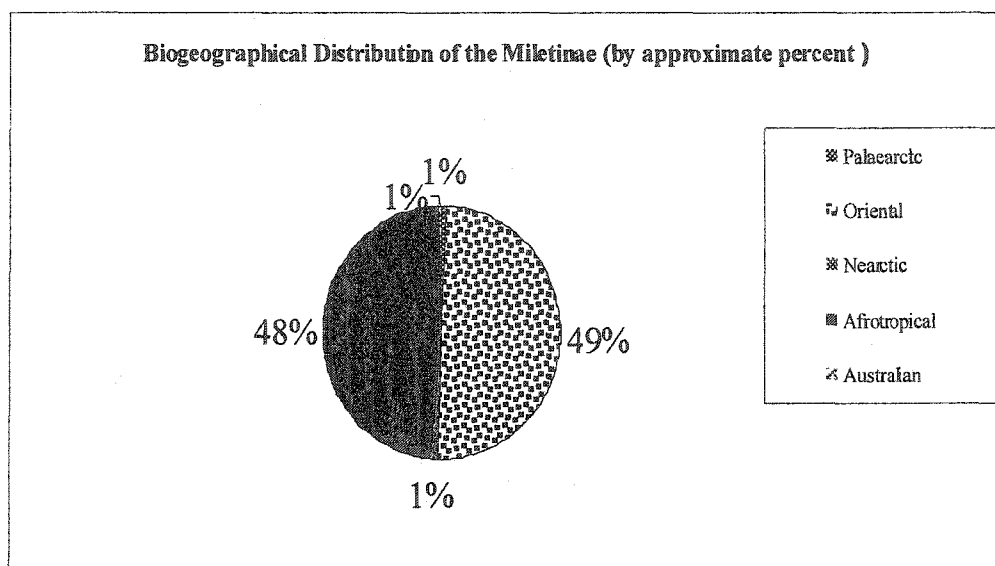


Figure 2.4. Biogeographical distribution of miletine tribes and genera by percent.

This small subfamily of about 150 species in 11 genera and four tribes (Table 2.1, Figure 2.3) displays its greatest radiation in the Oriental and Afrotropical regions, with minimal representation in the Nearctic, eastern Palearctic and Australian regions (one species each) (Table 2.1, Figure 2.4). This distribution may suggest an overall Gondwanan origin for the subfamily. *Liphyra brassolis* and *Taraka hamada*, respectively, may likely have dispersed from south-east Asia eastwards to Japan and Australia, and westward to north-eastern India. The Nearctic endemic, *Feniseca tarquinius*, may either represent an old Tertiary relic of a former Holarctic subtropical miletine fauna that was subsequently attenuated in the Palearctic through the glaciations or it may have entered America via the Bering Strait from eastern Asia (Fiedler 1991).

With respect to the tribes, the Miletini is completely Oriental with the exception of the genus *Megalopalpus* that consists of only four Afrotropical species. By contrast, the Lachnoncnemini is exclusively Afrotropical. The Spalgini has the widest distribution with representation in the Oriental, Afrotropical, Palearctic and Nearctic areas. However, it is primarily Oriental. It remains unclear why the tribe, and more specifically, *Spalgis* or *Taraka* did not manage to colonize the Australian mainland. The Liphyrini is Australian, Oriental, and Afrotropical, with all but one species endemic to Africa (Table 2.1, Figure 2.4). The Miletinae constitute a minor component of the lycaenid fauna (8%

each in southern Africa, Thailand and Peninsular Malaysia, and 11% in Borneo) (Corbet et al. 1992).

It is possible that the wider the range of a particular miletine taxon, the less selectional pressure there is on it to speciate. Most miletines do not migrate long distances and it is possible that gene flow is restricted, such that speciation events can occur. For phytophagous species, micro-allopatric speciation can occur where migration rates happen at the order of one insect per generation; however, selection must still overcome gene-flow (Berlocher & Feder 2002). Conceivably, the same pattern could hold for aphytophagous miletines. For those species that do have large distribution ranges, migration could counteract the effects of isolated gene pools. Rapoport's Rule (Stevens 1989) suggests that the genera with broader geographical ranges tend to belong to the higher latitudes. However, the occurrence of broad-range species in the high latitudes could be a consequence of successive glaciations, leaving only the most adaptable species behind (Cox & Moore 2000). These explanations might suggest the pattern seen among miletines, where the most widely distributed genera contain proportionately the fewest species (with the exception of *Lontalius*, which may not even be a valid genus).

As a rule, lycaenid species whose larvae have specific associations with ants tend to occur in highly isolated and fragmented populations (Pierce 1984; Smiley et al. 1988), often existing in single colonies in an extremely limited area. Examples include many Aphnaeini and *Lepidochrysops* species in Africa (Henning 1984; 1987), and *Acrodipsas illidgei* and *Paralucia pyrodiscus* in Australia (Samson 1987; 1989; Braby 1990). Pierce (1984) suggests that amplified opportunities for diversification (though not necessarily speciation) related to lycaenid-ant interactions can result from oviposition mistakes if the ant species is the cue, rather than plant-chemistry, for oviposition. More significantly, however, Pierce (1984) believes that the effects of population sizes are far more important. For the Miletinae, plant chemistry may not be as readily implicated since the plant is not the food-source, although chemistry may play a role in ovipositional cues. Furthermore, the vast majority of miletine-ant interactions appears to range between facultative and tolerated (Fiedler 1991). Table 2.2 lists obligate, facultative and myrmecoxenous Miletinae, as accrued from known life-histories (Figure 2.5) and extended to the whole subfamily circumspectly.

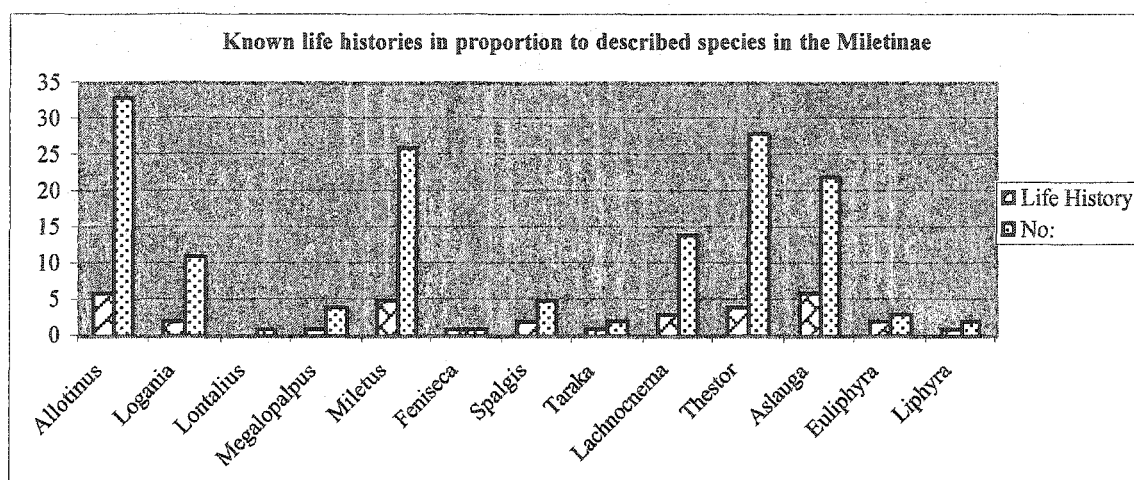


Figure 2.5. Known life histories in the Miletinae (i.e. no. of species with information on their ant-associates) in proportion to described species.

Table 2.2. Nature of myrmecophily in the Miletinae as deemed predominant in each genus.

| <i>Taxon</i> | <i>Nature of Myrmecophily</i> | <i>Number of included species</i> | <i>Known life histories</i> |
|---------------------|-------------------------------|-----------------------------------|-----------------------------|
| <i>Allotinus</i> | Facultative | 33 | 6 |
| <i>Logania</i> | Obligate | 11 | 2 |
| <i>Lontalius</i> | ? | 1 | 0 |
| <i>Megalopalpus</i> | Obligate | 4 | 1 |
| <i>Miletus</i> | Largely obligate | 26 | 5 |
| <i>Fenisecca</i> | Facultative | 1 | 1 |
| <i>Spalgis</i> | Facultative | 5 | 2 |
| <i>Taraka</i> | Facultative | 2 | 1 |
| <i>Lachnocnema</i> | Facultative | 13-14 | 3 |
| <i>Thestor</i> | Obligate | 28 | 4 |
| <i>Aslauga</i> | Facultative | 22 | 6 |
| <i>Euliphyra</i> | Obligate | 3 | 2 |
| <i>Liphya</i> | Obligate | 2 | 1 |

Predictions of Atsatt (1981) and Pierce and Elgar (1985) have resulted in the formulation of a number of criteria for the evolution of obligate and specific myrmecophily in the Lycaenidae. These include the following:

- a) Ecologically dominant ant species with highly predictable occurrence are preferred.
- b) Obligate myrmecophily normally arises in lycaenid taxa whose larvae search out shelter in ant nests for roosting, pupation or diapause.
- c) A persistent high enemy pressure reinforces the evolution of obligate associations.
- d) Caterpillars that prey upon ant brood have specific ant hosts.

As a corollary, Fiedler (1991) details conditions wherein obligate myrmecophily should rarely evolve. These are as follows:

- a) Rare ant species or ants with tiny colonies are not likely to host obligate myrmecophiles.
- b) Locations that have depauperate ant fauna such as islands, high latitudes or altitudes, rarely host obligately myrmecophilous lycaenids.
- c) Fewer obligate myrmecophiles are expected in tropical rainforests, where the ant fauna is extremely diverse.
- d) Widely distributed lycaenids, or those occurring in a broad range of ecological conditions, are unlikely to specialize on one particular host ant.

An examination of some of these criteria as they pertain to the Miletinae reveals the following:

- a) Obligately myrmecophilous genera are *Logania*, *Megalopalpus*, *Miletus*, *Thestor*, *Liphyra* and *Euliphyra* (Table 2.2). All but the genus *Thestor* occur within 20 degrees of the Equator and much of their habitat is tropical wet forest.
- b) The phenomenon of ecologically dominant ant species attending myrmecophiles appears to hold in the northern latitudes. Formicine ants are common in these areas (Cover personal communication), and facultatively myrmecophilous genera like *Feniseca tarquinius* are typically found in proximity to species of *Camponotus*, *Formica* and *Lasius* that tend their homopteran prey. However, the southern African genus *Thestor*, which has one of the largest radiations in the Miletinae (29 species), has not been reported to associate with any ant species other than *Anoplolepis custodiens* (Clark & Dickson 1971; Pringle et al. 1994; Heath and

Claassens 2000). Both *Liphyra brassolis* and *Euliphyra mirifica* associate exclusively with *Oecophylla*, the former with *O. smaragdina* (Dodd 1902; Johnson & Valentine 1986), the latter with *O. leucyanea* (Dejean 1991; Dejean and Beugnon 1996). Five species of *Miletus* are also associated with *Dolichoderus* (Dolichoderinae) in south-east Asia. *Logania marmorata* is obligately associated with *Hypoclinea* (Dolichoderinae), and *L. malayica* is obligately associated with the genus *Rhoptromyrmex* (Myrmicinae) (Fiedler 1993). *Allotinus*, whose range largely overlaps that of *Miletus*, is attended by a suite of ants belonging to different subfamilies (Fiedler 1993). However, *Allotinus unicolor* adults have been observed selectively to associate with *Anoplolepis longipes* (Maschwitz et al. 1985; Fiedler & Maschwitz 1989) and *Allotinus apries* pupates in *Myrmecaria lutea* nests (Maschwitz et al. 1988). While obligate myrmecophily is common in Australia and South Africa, and less so in the wet tropics, and rare in the temperate regions (Pierce 1987), the Miletinae needed to be treated differently since obligate aphytophagy is very different from obligate mutualistic myrmecophily. It is also important to treat the data with some circumspection, since representation of the subfamily is very sparse in the northern latitudes. It can be surmised that, of the life-histories elucidated, only the Miletinae run the gamut of ant associations between extremely obligate aphytophagy and myrmecoxeny.

Because the Miletinae are aphytophagous (i.e. feeding on non-plant sources), the question of a persistently high enemy pressure reinforcing the evolution of obligate associations is moot. In many cases, the obligate association is actually or potentially that of parasitism on the host-ant e.g. *Liphyra brassolis*, *Euliphyra mirifica*, *Thestor yildizae*, *Allotinus apries*. Aphytophagy is likely to have been derived from a mutualistic myrmecophilous template in the Lycaenidae, where only one to three species of otherwise phytophagous clades have adopted aphytophagy (Pierce et al. 2002). Since the mutualistic condition is so common within the Lycaenidae, selection for butterfly exploitation of attendant ants might evolve, given the right conditions.

Phylogenetic placement of tribes and genera within the Miletinae

Employing a phenetic analysis of 19 characters, both morphological and behavioral (larval foods), Eliot (1973) proposed a tentative classification and phylogeny of the subfamilies and tribes of Lycaenidae, which was subsequently modified by Scott and Wright (1990) and Eliot (in Corbet et al. 1992). This study, coupled with Eliot's (1986) revision of the Miletini, and Libert's (1994) treatment of *Aslauga*, forms the morphological foundation for existing hypotheses for relationships in the Miletinae (Figure 2.6).

Using molecular data, Pierce et al. (unpublished) have used the mitochondrial markers Cytochrome Oxidase I (COI) and Cytochrome Oxidase II (COII), as well as the nuclear gene Elongation Factor I-alpha (EF 1- α) to construct a phylogenetic relationship among the genera that comprise the Miletinae (Figure 2.7). These molecular results indicate strong concordance with the morphological propositions of Eliot (in Corbet et al. 1992) and Libert (1994).

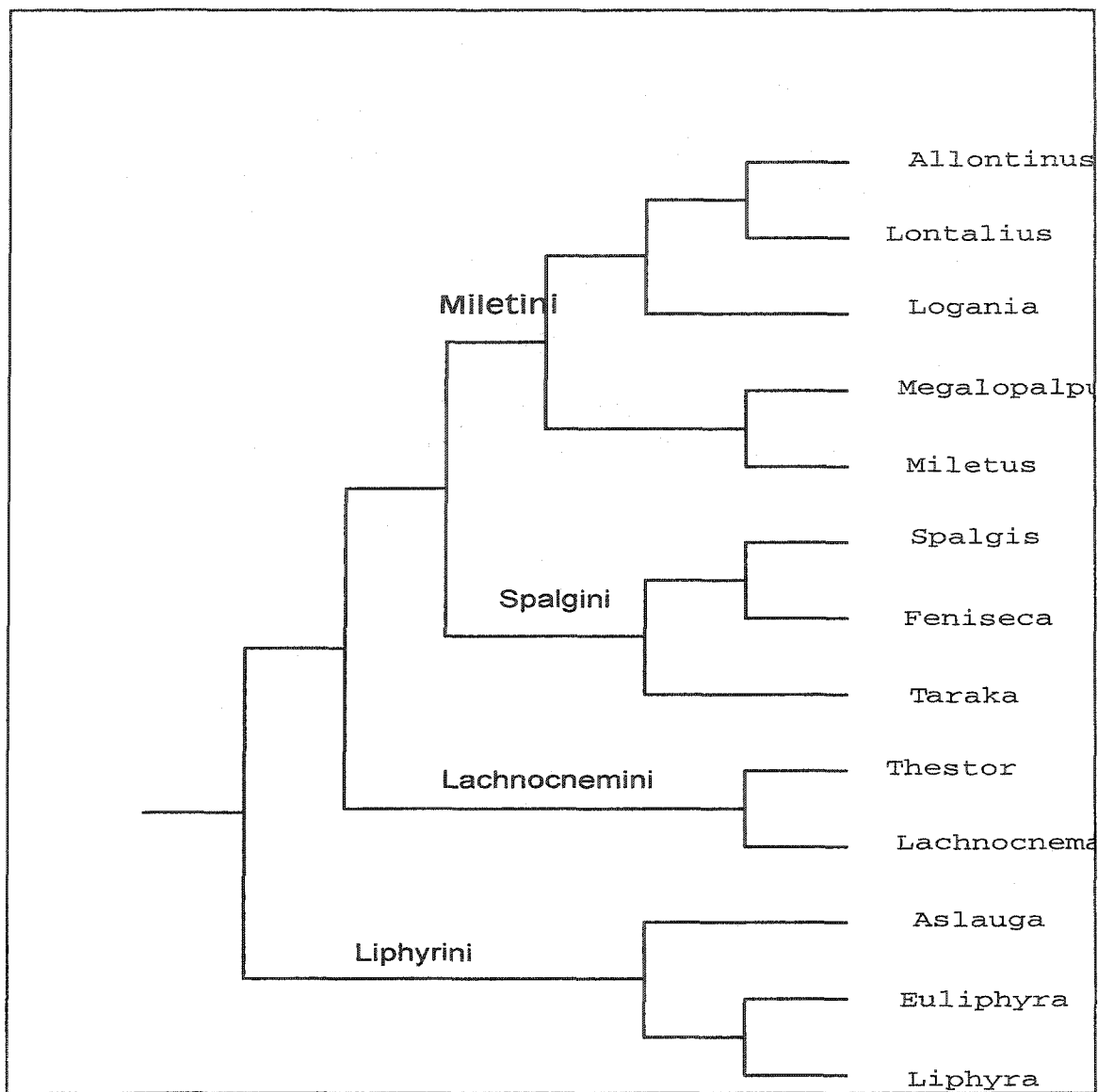


Figure 2.6. Tribal and generic relationships within the Miletinae, according to Eliot (1973; 1986); Corbet et al. (1992); and Libert (1994).

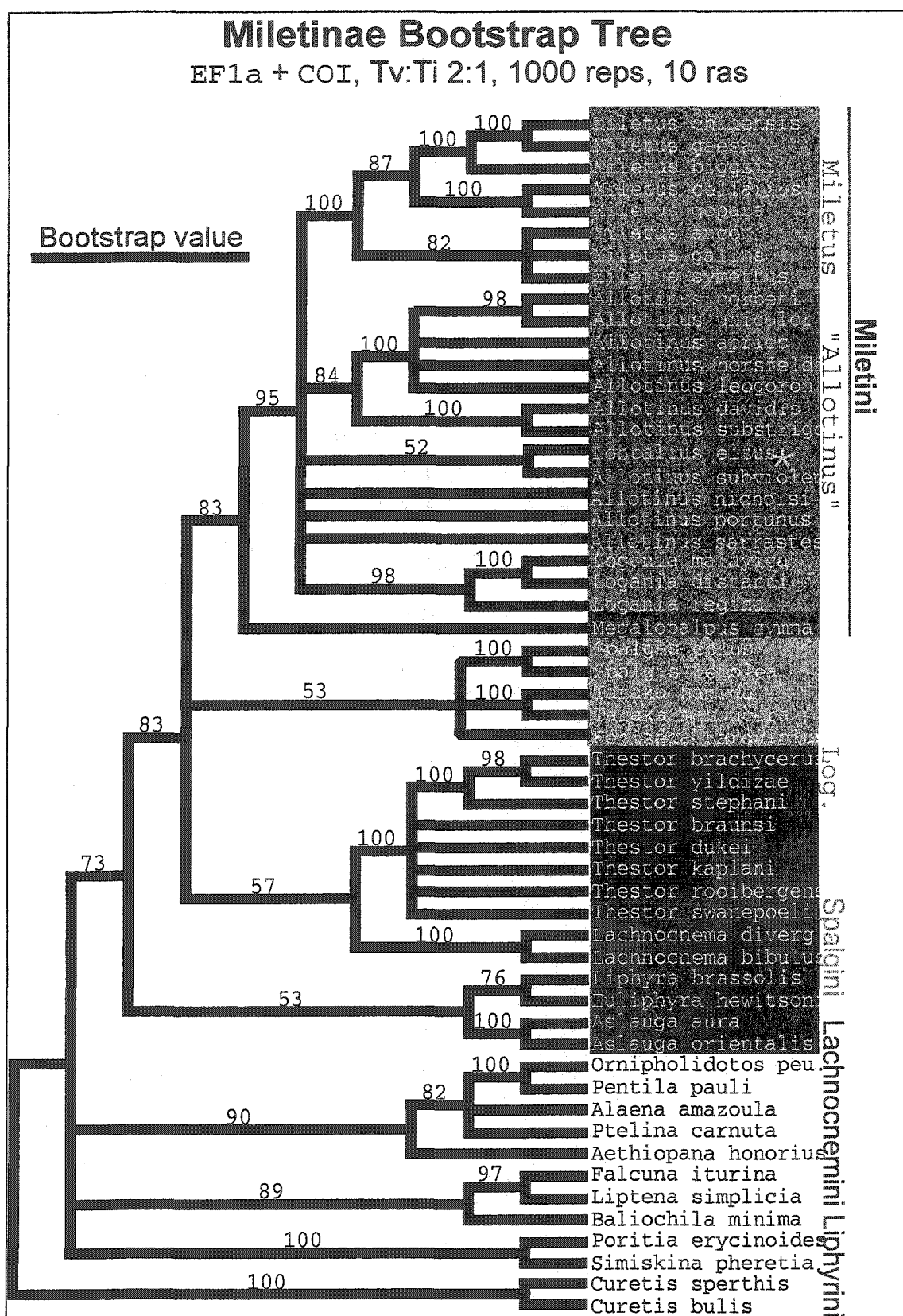


Figure 2.7. Molecular phylogeny of the Miletinae (Pierce et al. unpublished).

Aphytophagy in the Miletinae

Over 99% of the 160, 000 species that comprise the Lepidoptera eat plants (Strong et al. 1984; Common 1990; Powell et al. 1998; Pierce et al. 2002). Only about 250 species (Table 2.3) representing eight superfamilies are known to be obligate predators or parasites. Lepidopteran predators feed mainly on slow, soft-bodied scale insects, eggs of other insects, and ant brood, while lepidopteran parasites primarily attack other insects (Pierce 1995).

Table 2.3. Numbers of aphytophagous species per lepidopteran superfamily (from Pierce 1995).

| <i>Superfamily</i> | <i>Number of known species manifesting aphytophagy</i> |
|--------------------|--|
| a) Tineoidea | 34 |
| b) Gelechioidea | 39 |
| c) Tortricoidea | 6 |
| d) Zygaenoidea | 27 |
| e) Pyraloidea | 25 |
| f) Geometroidea | 17 |
| g) Noctuoidea | 37 |
| h) Papilionoidea | 81 |

The only aphytophagous species recorded in the Papilionoidea are in the Lycaenidae, including the Riodininae. The overwhelming majority is divided almost equally between the Lycaeninae and the Miletinae (Table 2.4) (Pierce et al. 2002).

Table 2.4. Lycaenid species with entomophagous life histories (from Pierce et al. 2002).

| <i>Confirmed entomophagous species (named if <3)</i> | <i>Species number</i> | <i>Food source^b</i> | <i>Ant associate (genus)</i> | <i>Distribution</i> |
|---|---------------------------|--------------------------------|----------------------------------|---------------------|
|---|---------------------------|--------------------------------|----------------------------------|---------------------|

Miletinae

Liphyrini

^b Bro = ant brood, Det = detritus, Eggs = ant eggs, Fung = algae, lichen & fungi, Hom = Homoptera, Hon = homopteran honeydew, Phy = plants, Tro = ant trophallaxis

Table 2.4. (continued)

| <i>Confirmed entomophagous species (named if <3)</i> | <i>Species number</i> | <i>Food source^b</i> | <i>Ant associate (genus)</i> | <i>Distribution</i> |
|--|-----------------------|--------------------------------|--|----------------------|
| <i>Liphyra brassolis</i> , <i>L. grandis</i> | 2/2 | Bro | <i>Oecophylla</i> | Australian, Oriental |
| <i>Euliphyra leucyana</i> , <i>E. mirifica</i> | 2/2 | Tro | <i>Oecophylla</i> | African |
| <i>Aslauga</i> (9 spp.) Miletini | 20/20 | Hom | | African |
| <i>Allotinus</i> (6 spp.) | 30/30 | Hom | <i>Anoplolepis</i> , <i>Crematogaster</i> , <i>Oecophylla</i> , <i>Technomyrmex</i> | Oriental |
| <i>Logania malayica</i> , <i>L. marmorata</i> | 10/10 | Hom/Hon/Tro | <i>Leptothorax</i> | Oriental |
| <i>Lontalius</i> (0 spp.) | 1/1 | ? | | |
| <i>Megalopalpus zymna</i> | 4/4 | Hom | <i>Pheidole</i> | Afrotropical |
| <i>Miletus</i> (5 spp.) | 20/20 | Hom | <i>Crematogaster</i> , <i>Dolichoderus</i> , <i>Pheidole</i> , <i>Polyrachis</i> | Oriental |
| Spalgini | | | | |
| <i>Spalgis epius</i> , <i>S. lemolea</i> | 5/5 | Hom | <i>Crematogaster</i> , <i>Oecophylla</i> | African, Oriental |
| <i>Taraka hamada</i> | 2/2 | Hom/Hon | | Palearctic, Oriental |
| <i>Feniseca tarquinius</i> | 1/1 | Hom/Hon | <i>Camponotus</i> , <i>Formica</i> , <i>Lasius</i> , <i>Myrmica</i> | Nearctic |
| Lachnocnemini | | | | |
| <i>Lachnocnema bibulus</i> , <i>L. brimo</i> , <i>L. durbani</i> | 12/12 | Hom/Hon/Bro | <i>Camponotus</i> , <i>Crematogaster</i> , <i>Pheidole</i> | African |
| <i>Thestor basutus</i> , <i>T. protumnus</i> , <i>T. yildizae</i> | 27/27 | Hom/Tro/Det | <i>Anoplolepis</i> | African |

^b Bro = ant brood, Det = detritus, Eggs = ant eggs, Fung = algae, lichen & fungi, Hom = Homoptera, Hon = homopteran honeydew, Phy = plants, Tro = ant trophallaxis

Table 2.4. (continued)

| <i>Confirmed entomophagous species (named if <3)</i> | <i>Species number</i> | <i>Food source^b</i> | <i>Ant associate (genus)</i> | <i>Distribution</i> |
|--|-----------------------|--------------------------------|---|---------------------|
| Lycaeninae | | | | |
| Theclini | | | | |
| Arhopaliti | | | | |
| <i>Arhopala wildei</i> | 1/120 | Bro | <i>Polyrachis</i> | Australian |
| Luciiti | | | | |
| <i>Acrodipsas aurata</i> , <i>A. brisbanensis</i> , <i>A. cuprea</i> , <i>A. illidgei</i> , <i>A. myrmecophila</i> | 9/9 | Bro | <i>Crematogaster</i> , <i>Papyrius</i> | Australian |
| Ogyriti | | | | |
| <i>Ogyris idmo</i> , <i>O. subterrestris</i> | 2/14 | Tro? | <i>Camponotus</i> | Australian |
| Thecliti | | | | |
| <i>Shirozua jonasi</i> | 1/1 | Tro/Hom | <i>Lasius</i> | |
| Zesiiti | | | | |
| <i>Zesius chrysomallus</i> | 1/1 | Phy/Bro? | <i>Oecophylla</i> | South Asian |
| Aphnaeini | | | | |
| <i>Aloeides pallida grandis</i> | 1/50 | Eggs/Phy | <i>Lepisiota</i> | African |
| <i>Aphnaeus adamsi</i> | 1/20 | Tro/Fung | <i>Crematogaster</i> | African |
| <i>Argyrospodes argyraspis</i> | 1/1 | ? | ? | African |
| <i>Axiocerses harpax</i> | 2/10 | Tro | <i>Crematogaster</i> | African |
| <i>Chloroselas pseudozeritis umbrosa</i> | 1/14 | Tro? | <i>Crematogaster</i> | African |
| <i>Chrysoritis dicksoni</i> | 1/58 | Tro | <i>Crematogaster</i> | African |
| <i>Cigaritis [Apharitis] acamas</i> , <i>C. [Spindasis] nyassae</i> , <i>C. [Spindasis] takanonis</i> | 3/65 | Tro/Bro | <i>Crematogaster</i> | African, Japanese |

^b Bro = ant brood, Det = detritus, Eggs = ant eggs, Fung = algae, lichen & fungi, Hom = Homoptera, Hon = homopteran honeydew, Phy = plants, Tro = ant trophallaxis

Table 2.4. (continued)

| <i>Confirmed entomophagous species (named if <3)</i> | <i>Species number</i> | <i>Food source^b</i> | <i>Ant associate (genus)</i> | <i>Distribution</i> |
|--|-----------------------|--------------------------------|--|-----------------------|
| <i>Trimenia agyropilaga</i> , <i>T. wallengrenii</i> , <i>T.</i> (<i>Argyrocupha</i>) <i>malagrida</i> | 5/5 | Tro/Bro | <i>Anoplolepis</i> | African |
| Polyommagini | | | | |
| Lycaenesthiti | | | | |
| <i>Anthene levis</i> | 1/90 | Tro | ? | African |
| <i>Triclema lamias</i> | 1/20 | Hom | ? | African |
| Niphanditi | | | | |
| <i>Niphanda fusca</i> | 1/10 | Tro | <i>Camponotus</i> sp. | Oriental/Palearctic |
| Polyommati | | | | |
| <i>Chilades lajus</i> | 1/17 | Hom | ? | Japanese |
| <i>Lepidochrysops</i> (11 spp.) | 126/126 | Phy/Bro/Tro | <i>Camponotus</i> | African |
| <i>Maculinea</i> (6 spp.) | 6/6 | Phy/Bro/Tro | <i>Myrmica</i> , <i>Aphaenogaster</i> | European/ Oriental |
| <i>Phengaris daitozana</i> , <i>P. atroguttata</i> | 2/2 | Phy/Bro | <i>Myrmica</i> | Oriental |
| Riodininae | | | | |
| Nymphidini | | | | |
| <i>Setabis lagus</i> | ?/27 | Hom | ? | Neotropical |
| Eurybiini | | | | |
| <i>Alesa amesis</i> | ?/5 | Hom | <i>Camponotus femoratus</i> | Neotropical |
| Lemoniini | | | | |
| <i>Audre aurina</i> | ?/31 | Tro? | <i>Camponotus</i> , <i>Solenopsis</i> | Neotropical |

^b Bro = ant brood, Det = detritus, Eggs = ant eggs, Fung = algae, lichen & fungi, Hom = Homoptera, Hon = homopterian honeydew, Phy = plants, Tro = ant trophallaxis

Constraints in shifting to aphytophagy

Aphytophagy requires specialization. Feeding on Homoptera that produce honeydew is contingent upon an ability to appease ants that are in competition for the same resources (Malicky 1970; DeVries & Baker 1989). Consumption of homopterans requires not just the ability to appease ants that may be tending the homopterans, but adaptations for finding homopteran prey, some of which can be patchy and ephemeral. By contrast, feeding on ant brood requires extreme chemical and morphological specialization to find and penetrate ant nests (Thomas et al. 1989; Elmes et al. 1991a; 1991b; 1994; Thomas & Wardlaw 1992; DeVries et al. 1993). Among the Lepidoptera consuming either Homoptera or ants, there must be an appropriate digestive physiology to develop on one of two prey types (Dadd 1983). Those caterpillars that feed on ant regurgitations must be able to penetrate the ant nest by means of chemical camouflage (*Maculinea*) or brute force (*Liphyra*); furthermore, they must be able to mimic the appropriate behavioral cues to solicit regurgitations from their host ants (Holldobler & Wilson 1990).

Pierce (1995) discussed possible preadaptations leading to the evolution of carnivory in the Lepidoptera. Within the Miletinae, larvae protect themselves from ant attack in a variety of ways, as given below:

- a) The spinning of a silken web that functions as a shelter while the larva feeds on homopteran prey (*Spalgis*, *Taraka*, *Feniseca*).
- b) The adults of many species of homopterophagous Lycaenidae have unusually long and sclerotized legs and abdomens, which may protect them against attacks by ants when ovipositing near their homopteran prey (*Allotinus*, *Lachnocnema*).
- c) Adults of species that eclose within the nests of host ants are often cloaked in deciduous scales that aid them in safely exiting from the nest (*Liphyra*).
- d) The larvae of myrmecophilous lycaenids are known to possess unusually thick cuticles (*Liphyra*).

Apart from these defenses, it is possible that adults may secrete volatile compounds that protect them from ant attacks, although none of these have been identified (Pierce 1995).

Among the Miletinae, many species feed on plant sap (e.g. *Logania*) (Fielder 1993; 1996). The sap that is derived from extra-floral nectaries may be similar in composition to the honey-dew that aphids excrete and adults of many homopterophagous butterflies consume. Furthermore, many members of the Lycaenidae are well known for cannibalistic tendencies under field and laboratory conditions alike, both aphytophagous species like *Lachnocnema bibulus* (Cottrell 1984), *Feniseca tarquinius* (Clark 1926), *Logania malayica* (Fielder 1993) and phytophagous species such as *Zesius chrysomallus* (Hinton 1951). The immediate question arises: can the same conditions that predispose a tendency to cannibalism be invoked to explain a shift to predatory behavior? A review of phylogenetic relationships does not readily provide an answer, and it is likely that ecological conditions play a significant role (for instance, laboratory conditions in which caterpillars can be starved for typical food, might result in atypical behavior).

Maschwitz et al. (1988) propose that with respect to the Homoptera, feeding on the Auchenorrhyncha was derived from preying on the Sternorrhyncha. They speculate that such species as *Logania malayica* represent the ancestral pattern, feeding mainly on ant-attended aphids. In contrast, many species of *Miletus* and *Allotinus* are far more catholic in their diet. I collected life-history information where available for the Miletinae and matched food source to taxon (Table 2.5).

Table 2.5. Correlation of food source to miletine feeder.

| <i>Food Source</i> | <i>Taxon</i> |
|--------------------|---|
| 1) Sternorrhyncha | <i>Aslauga</i> (Liphyrini), <i>Lachnocnema</i> , <i>Thestor</i> (<i>Lachnocnemini</i>), <i>Spalgis</i> , <i>Taraka</i> , <i>Feniseca</i> (<i>Spalgini</i>), <i>Allotinus</i> , <i>Miletus</i> (<i>Miletini</i>) |
| 2) Auchenorrhyncha | <i>Aslauga</i> (Liphyrini), <i>Lachnocnema</i> (<i>Lachnocnemini</i>), <i>Allotinus</i> , <i>Logania</i> , <i>Megalopalpus</i> (<i>Miletini</i>) |
| 3) Ant-brood | <i>Liphyra</i> (Liphyrini), <i>Allotinus</i> , <i>Miletus</i> (<i>Miletini</i>), <i>Thestor</i> (<i>Lachnocnemini</i>) |
| 4) Regurgitations | <i>Euliphyra</i> (Liphyrini), <i>Lachnocnema</i> , <i>Thestor</i> , (<i>Lachnocnemini</i>), <i>Logania</i> (<i>Miletini</i>) |
| 5) Detritivory | <i>Thestor</i> (<i>Lachnocnemini</i>) |
| 6) Cannibalism | <i>Aslauga</i> (Liphyrini), <i>Feniseca</i> (<i>Spalgini</i>) |
| 7) Plant Sap | <i>Lachnocnema</i> (<i>Lachnocnemini</i>), <i>Logania</i> (<i>Miletini</i>) |

Pierce et al. (unpublished) present a more generalized picture (Figure 2.8). We treat feeding on the Auchenorrhyncha and Stenorrhyncha together under homopterophagy, resulting in an overwhelming pattern of homopterophagy in the Miletinae. In this analysis, the following sequence of events is suggested:

- a) The Liphyrinae are the basal clade, and based upon this, ancestral miletines may have been both myrmecophagous and homopterophagous/ trophallactic.
- b) Homopterophagy appears to be ancestral for all other miletine groups.
- c) Feeding on ant brood in addition to Homoptera appears to be derived in these groups.
- d) A comparison of Sternorrhyncha versus Auchenorrhyncha shows no strong patterns.

This treatment does suggest considerable phylogenetic constraint in feeding patterns in the Miletinae.

Aphytophagy in the Miletinae

Consensus tree using nodes with >70% bootstrap support

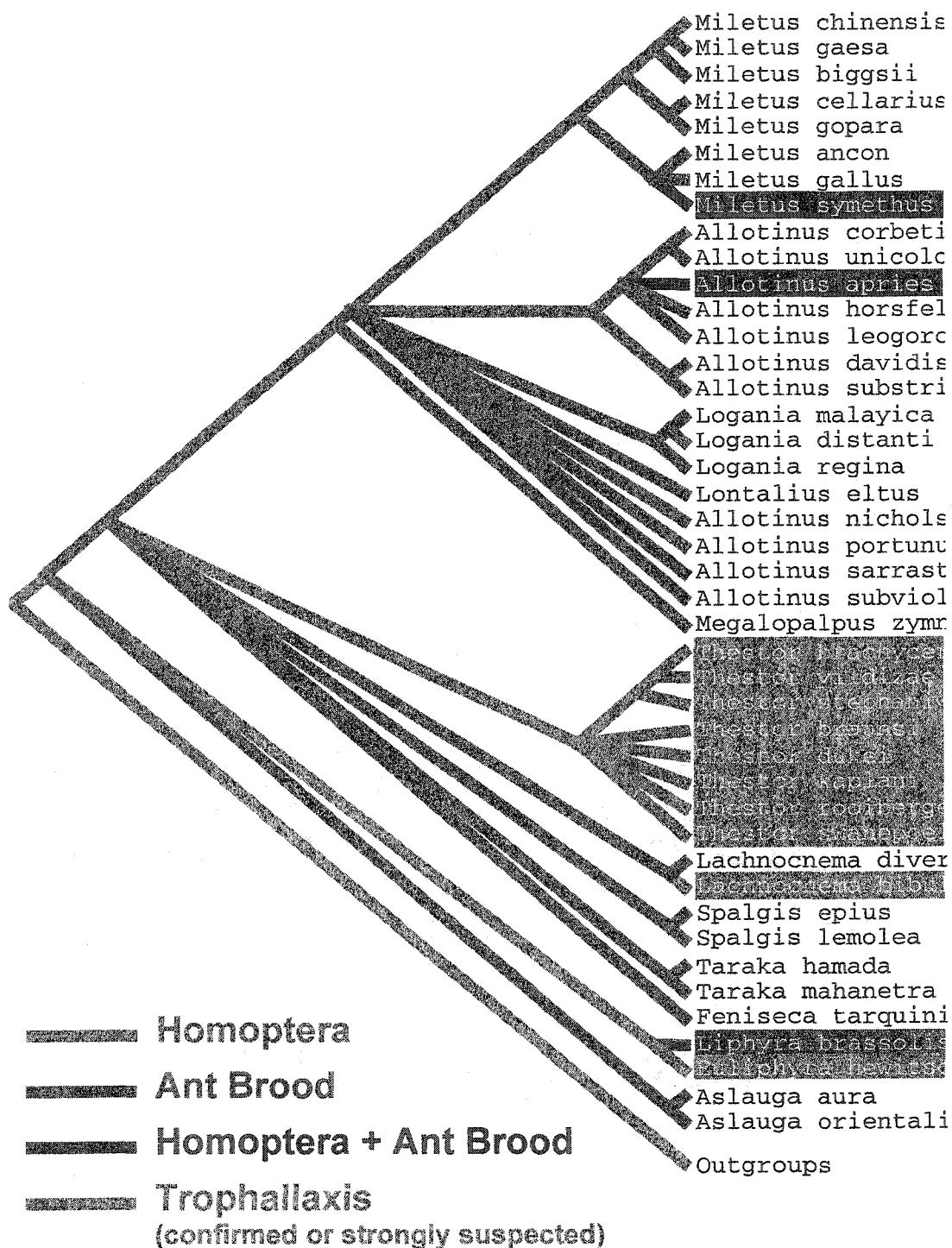


Figure 2.8. Feeding patterns in the Miletinae (Pierce et al. unpublished).

Brief history of research in the Miletinae

Aphytophagy in the Miletinae was first described by Riley (1886) in a one page report to the journal *Science* on *Feniseca tarquinius*. In the same year, Edwards (1886) reported the life history of *Feniseca tarquinius* in greater detail in the *Canadian Entomologist* based largely upon the observations of Miss Emily Morton. Before this time, all miletines were considered phytophagous, based on speculative descriptions pertaining to this fact (Grote 1869; Scudder 1897). After the observation that aphytophagy does occur, the life histories of representatives of several other miletine genera, *Spalgis*, *Taraka*, *Liphyra*, *Gerydus* (now *Miletus*), *Aslauga*, *Euliphyra* and *Megalopalpus* were elucidated within 30 years (Aitken 1894; Tsuchida 1898; Dodd 1902; Kershaw 1905; Lamborn 1913; Roepke 1919; Farquharson 1921; Clark 1926). Of these, the elaboration of the life-history of *Taraka hamada* bears particular mention: Iwase (1953) reported that when Dr. Albert Koebele, the famous biocontrol expert from the United States visited Japan in 1894-95 en route to Australia, he related the habit of *Feniseca tarquinius* to T. Tsuchida, who, based upon this information, soon determined (1898), the life history of the Palaearctic *Taraka hamada*, which almost completely parallels that of *Feniseca*.

After initial information on life-history had accrued for most miletine genera, the patterns of the aphytophagous habit were investigated by a series of workers (Green 1902; Clark 1926; Jackson 1937; Hinton 1951; Cottrell 1984; Fiedler 1991; Pierce 1995). Furthermore, much attention was and continues to be paid to various species of the Miletinae from the perspective of economic entomology, because the larvae could potentially be used as bio-control agents. Ackery (1990) described how both *Spalgis epius* and *Spalgis lemolea* were excellent controls for homopteran species of *Phenacoccus*, *Planococcus*, *Pseudococcus*, and *Ferrisia*. He suggested that *Aslauga* might also be useful in economic pest control. *Feniseca tarquinius* was once considered in this light; Brower (1947) reported that whole colonies of the destructive balsam woolly aphid, *Adelges piceae* were preyed upon by a dozen larvae of *Feniseca tarquinius*. This was the first record of such a phenomenon, and little has emerged since then from this line of pursuit for *Feniseca tarquinius*, presumably because adelgids are not the preferred prey for the species.

Life-history summary for the Miletinae

Table 2.6 summarizes all known life-history information on the Miletinae, by species.

Table 2.6. Consolidated life-history information for the Miletinae.

| <i>Species</i> | <i>Food substrate</i> | <i>Myrmecophily</i> | <i>Associated ants with subfamily</i> | <i>References</i> |
|-------------------------------|--|---------------------------|---|--|
| <i>Liphyra brassolis</i> | <i>Oecophylla</i> Brood (direct observation) | Obligate | <i>Oecophylla smaragdina</i> (Formicinae) | Dodd 1902; Johnson & Valentine 1986; Cottrell 1987. |
| <i>Liphyra grandis</i> | <i>Oecophylla</i> Brood (direct observation) | Obligate | <i>Oecophylla smaragdina</i> (Formicinae) | Parsons 1991. |
| <i>Euliphyra mirifica</i> | <i>Oecophylla</i> Regurgitations + prey items of hosts (direct observation) | Obligate | <i>Oecophylla longinoda</i> (Formicinae) | Lamborn 1913; Hinton 1951; Cottrell 1984; Dejean 1991; Dejean & Beugnon 1996. |
| <i>Euliphyra leucyana</i> | <i>Oecophylla</i> regurgitations + prey items of hosts (direct observation) | Obligate | <i>Oecophylla longinoda</i> (Formicinae) | Kielland 1990; Dejean 1991. |
| <i>Aslauga lamborni</i> | Membracidae Coccidae (direct observation) | Myrmecoxenous Only TOs | <i>Crematogaster</i> sp. (indifferent) (Myrmicinae) | Lamborn 1913; van Someren 1974; Cottrell 1984; Ackery & Rajan 1990. |

Table 2.6.
(continued)

| <i>Species</i> | <i>Food substrate</i> | <i>Myrmecophily</i> | <i>Associated ants with subfamily</i> | <i>References</i> |
|-----------------------------|--|--------------------------------|---|---|
| <i>Aslauga purpurascens</i> | Membracidae Coccidae (lab) Psyllidae (lab) (direct observation) | Myrmecoxenous Only TOs | | Boulard 1968; Cottrell 1981. |
| <i>Aslauga latifurca</i> | Membracidae Coccidae (direct observation). Note: Cannibalistic | Myrmecoxenous ? Only TOs | | Jackson 1937; Cottrell 1981; Ackery & Rajan 1990. |
| <i>Aslauga atrophifurca</i> | Homoptera (direct observation) | Myrmecoxenous Only TOs ? | | Cottrell 1984; Villet 1986. |
| <i>Aslauga orientalis</i> | Coccidae (direct observation) | Myrmecoxenous (Only TOs) | | Cottrell 1981. |
| <i>Aslauga vininga</i> | Coccidae Pseudococcidae (direct observation) | Myrmecoxenous ? (Only TOs) | <i>Crematogaster</i> sp. (indifferent) (Myrmicinae) | Lamborn 1913; Cottrell 1984; Ackery & Rajan 1990. |
| <i>Spalgis epius</i> | Coccidae (direct observation) | Myrmecoxenous | | Aitken 1894; Green 1902; Misra 1920; Cottrell 1984; Scott 1986. |
| <i>Spalgis lemolea</i> | Coccidae Pseudococcidae | Myrmecoxenous | <i>Crematogaster</i> sp., (Myrmicinae) <i>Oecophylla</i> <i>longinoda</i> , (Formicinae) (all indifferent to homopterans) | Lamborn 1913; Boulard 1968; Cottrell 1984. |
| <i>Spalgis substrigata</i> | Coccidae (Direct observation) | | | Smith 1914. |

Table 2.6.
(continued)

| <i>Species</i> | <i>Food substrate</i> | <i>Myrmecophily</i> | <i>Associated ants with subfamily</i> | <i>References</i> |
|-------------------------------|--|--|---|---|
| <i>Fenisea tarquinius</i> | Pemphigidae (Homoptera-feeding, direct observation) | Myrmecoxenous | | Riley 1886; Edwards 1886; Scott 1986; Klassen et al. 1989. |
| <i>Taraka hamada</i> | Hormaphididae+honey dew+siphon secretions (direct observation) | Myrmecoxenous | | Cottrell 1984; Banno 1990. |
| <i>Miletus chinensis</i> | Aphidoidea (Direct observation) | Myrmecoxenous (Maybe highly myrmecophilous?) | <i>Dolichoderus bituberculatus</i> (Dolichoderinae) <i>Polyrhachis dives</i> (?)(Formicinae) | Cottrell 1984. |
| <i>Miletus boisduvali</i> | Aphidoidea Coccidae (Direct observation) | Myrmecoxenous (Maybe highly myrmecophilous?) | <i>Dolichoderus</i> sp., (Dolichoderinae) <i>Polyrhachis</i> sp. (Formicinae) | Roepke 1919; Cottrell 1984. |
| <i>Miletus biggsii</i> | Hormaphididae Coccidae (Direct observation) | Myrmecoxenous (Maybe obligate?) | <i>Dolichoderus</i> sp. (Dolichoderinae) | Maschwitz et al. 1985; 1988. |
| <i>Miletus symethus</i> | Coccidae <i>Dolichoderus</i> brood (Direct observation) | Myrmecoxenous (Maybe obligate?) | <i>Dolichoderus</i> sp. (Dolichoderinae) | Roepke 1919; Eliot 1980. |
| <i>Miletus nymphis</i> | Coccidae (Direct observation) | Myrmecoxenous (Maybe highly myrmecophilous) | | Maschwitz et al. 1988. |
| <i>Allotinus unicolor</i> | Hormaphididae Psyllidae? Membracidae? (Direct observation) | Myrmecoxenous (Maybe highly myrmecophilous?) | <i>Anoplolepis longipes</i> (indifferent to oviposition) (Formicinae) | Maschwitz et al. 1985; Fiedler & Maschwitz, 1989. |

Table 2.6.
(continued)

| <i>Species</i> | <i>Food substrate</i> | <i>Myrmecophily</i> | <i>Associated ants with subfamily</i> | <i>References</i> |
|-------------------------------|---|---------------------|---|---|
| <i>Allotinus subviolaceus</i> | Membracidae (Direct observation) | Myrmecoxenous | <i>Anoplolepis longipes</i> (indifferent) (Formicinae) | Maschwitz et al. 1985; Maschwitz et al. 1988. |
| <i>Allotinus major</i> | Membracidae (Direct observation) | Myrmecoxenous | <i>Anoplolepis longipes</i> (indifferent to oviposition) (Formicinae) | Kitching 1987. |
| <i>Allotinus davidis</i> | Aphidoidea (Direct observation) | Myrmecoxenous | <i>Crematogaster difformis</i> (indifferent) (Myrmicinae) | Maschwitz et al. 1985; Maschwitz et al. 1988. |
| <i>Allotinus substrigosus</i> | Hormaphididae (Direct observation) | Myrmecoxenous | <i>Crematogaster</i> sp., (adult), (Myrmicinae) <i>Technomyrmex</i> sp. (indifferent) (Dolichoderinae) | Maschwitz et al. 1985; Maschwitz et al. 1988; Schutze 1990. |
| <i>Allotinus apries</i> | Coccidae (L1) <i>Myrmecaria</i> brood (Direct observation) Ant Brood (Inferred) | Obligate ? | <i>Myrmecaria lutea</i> (Myrmicinae) | Maschwitz et al. 1988. |
| <i>Logania malayica</i> | Aphididae, Coccidae, Membracidae - honeydew, extrafloral nectar, cannibalism, (Direct observation), Ant regurgitations (Inferred) | Obligate | <i>Rhoptromyrmex wroughtonii</i> (Myrmicinae) | Maschwitz et al. 1988; Fiedler 1993; Fiedler 1996. |
| <i>Logania marmorata</i> | Membracidae, Extra- floral nectar, honeydew (Direct observation) | Obligate | <i>Hypoclinea</i> sp. (Dolichoderinae) | Fiedler 1993; Fiedler 1996. |

Table 2.6.
(continued)

| <i>Species</i> | <i>Food substrate</i> | <i>Myrmecophily</i> | <i>Associated ants</i> <i>with subfamily</i> | <i>References</i> |
|--|---|------------------------------------|--|---|
| <i>Megalopalpus</i> <i>zymna</i> | Membracidae Jassidae (Direct observation) | Myrmecoxenous | <i>Pheidole aurivillii</i> (indifferent) (Myrmicinae) | Lamborn 1913; Cottrell 1984; Ackery 1990. |
| <i>Lachnocnema</i> <i>bibulus</i> | Jassidae Membracidae Psyllidae + honeydew + <i>Camponotus</i> regurgitations (Direct observation) | Myrmecoxenous (Maybe obligate?) | <i>Crematogaster</i> sp., (Myrmicinae) <i>Pheidole</i> sp. (indifferent) (Myrmicinae) <i>Camponotus</i> <i>acvapimensis</i> (Formicinae) <i>Camponotus</i> <i>maculatus</i> (Formicinae) | Farquharson 1921; Clark 1940, Cripps & Jackson 1940; van Someren 1974; Cottrell 1984. |
| <i>Lachnocnema</i> <i>brimo</i> | Membracidae Psyllidae (Direct observation) | Myrmecoxenous? | <i>Camponotus</i> sp. (indifferent) (Formicinae) | Ackery 1990; Ackery & Rajan 1990. |
| <i>Lachnocnema</i> <i>durbani</i> | Coccidae (lab) Membracidae (lab) (Direct observation) | Myrmecoxenous? | | Ackery & Rajan 1990; Larsen 1991. |
| <i>Thestor</i> <i>basutus</i> | Ant brood? (Inferred) Detritus | Obligate | <i>Anoplolepis</i> <i>custodiens</i> (Formicinae) | Clark & Dickson 1971; Heath & Claassens 2000. |
| <i>Thestor</i> <i>obscurus</i> (T. <i>yildizae</i>) | Ant brood? (Inferred) Trophallaxis (Direct Observation) | Obligate? | <i>Anoplolepis</i> <i>custodiens</i> (Formicinae) | Claassens & Dickson 1980; Heath & Claassens 2000. |
| <i>Thestor</i> <i>brachycerus</i> | Ant brood? (Inferred) | {Obligate?} | <i>Anoplolepis</i> <i>custodiens</i> (Formicinae) | Clark & Dickson 1971. |
| <i>Thestor</i> <i>dukei</i> | Ant brood? (Inferred) | {Obligate?} | <i>Anoplolepis</i> <i>custodiens</i> (Formicinae) | Clark & Dickson 1971. |

Table 2.6.
(continued)

| <i>Species</i> | <i>Food substrate</i> | <i>Myrmecophily</i> | <i>Associated ants with subfamily</i> | <i>References</i> |
|------------------------------|---|---------------------|---|---|
| <i>Thestor rileyi</i> | Homoptera (Direct Observation) Ant brood? (Inferred) | {Obligate?} | <i>Anoplolepis custodiens</i> (Formicinae) | Clark & Dickson 1960; Clark & Dickson 1971. |
| <i>Thestor holmesi</i> | Ant brood? (Inferred) | Obligate | <i>Anoplolepis custodiens</i> (Formicinae) | Clark & Dickson 1971. |
| <i>Thestor protumnus</i> | Coccidae (Direct Observation) Ant brood? (Inferred) | {Obligate?} | <i>Anoplolepis custodiens</i> (Formicinae) | Clark & Dickson 1971; Migdoll 1988. |

SECTION 3

PHYLOGENY AND LIFE HISTORY EVOLUTION OF THE GENUS *THESTOR* (LEPIDOPTERA, MILETINAE)

The genus *Thestor* (Hubner 1819) (Lycaenidae: Miletinae) is endemic to southern Africa, with all but one species confined to South Africa (Clark & Dickson 1971; Claassens & Dickson 1980; Pringle et al. 1994), in particular, the Western, Eastern and Northern Cape Provinces (Pringle et al. 1994). As with other miletines, the genus is aphytophagous (Cottrell 1984; Fiedler 1991; Pierce 1995), with the primary food source appearing to be ant-regurgitations, and homopteran prey (Clark & Dickson 1971, Migdoll 1988, Heath & Claassens 2000). *Thestor* is found in a variety of habitats and altitudes, ranging from sea-level (*Thestor malagas*) to an altitude of up to 1000 m (*T. penningtoni*) (Pringle et al. 1994). Females oviposit on almost any substrate (Heath & Claassens 2000), customarily close to nests of the formicine ant species, *Anoplolepis custodiens*, with which the genus invariably appears to be associated (Pringle et al. 1994).

The first reference to *Thestor* in the primary literature is by Carl von Linné in 1764, when he described the first species from the group under the name *Papilio protumnus*. It was only in 1819 that the name *Thestor* was applied to the genus by Hubner. The genus was also briefly known as *Arrugia* (Wallengren 1872). No information on the life-history of the genus or the subfamily as a whole was forthcoming for nearly a century thereafter, until the pioneering discoveries of the aphytophagous nature of the Nearctic genus *Feniseca* by Riley (1886) and Edwards (1886). These findings sparked a number of remarkable life history studies in the subfamily Miletinae over the next forty years, elucidating the aphytophagous strategies of the genera *Spalgis*, *Liphyra*, *Taraka*, *Miletus* (*Gerydus*), *Megalopalpus*, *Lachnocnema*, *Aslauga* and *Euliphyra*, (for review, please see Cottrell 1984, and Section 2) with Clark (1926) even opining (correctly) that members of the genus *Allotinus* might be likewise inclined. The first insights, by Clark and Dickson, into the life-history of *Thestor*, however, did not occur until 1960. In contrast, the life-history of a species of *Lachnocnema* (*Lachnocnema bibulus*), the only clade other than *Thestor* in the tribe Lachnocnemini, was determined by Lamborn as early as 1913.

The species of *Thestor* have been placed, traditionally and informally, into two groups, the yellow and the black *Thestor* (Table 3.1), based upon whether the insect is in body and wing color, either yellow to white, or dark-brown to black. With more species being described and assigned to one or the other of these groups, the validity of the distinctions on the basis of color alone became somewhat questionable especially when some species (eg. *T. murrayi*) were possessed of both light and dark forms (Heath personal communication). Most recently, Heath and Pringle (personal communication) have undertaken a comprehensive revision of the genus based chiefly upon genitalic studies and foreleg examination. Input from other sources, including SEM examination of eggs have yielded so much variation that they have been of limited use. Based on these studies, Heath and Pringle have suggested the following informal groupings:

- a) The basutus group: *T. basutus basutus*, *T. basutus capeneri*
- b) The protumnus group: *T. protumnus protumnus*, *T. protumnus aridus*, *T. protumnus mijburghi*, *T. dryburghi*, *T. terblanchei*
- c) The rossouwi group: *T. rossouwi*, *T. swanepoeli*, *T. murrayi*, *T. strutti*
- d) The dicksoni group: *T. dicksoni dicksoni*, *T. dicksoni calviniae*, *T. dicksoni warreni*, *T. malagas*
- e) The montanus group: *T. montanus*, *T. vansoni*, *T. rooibergensis*, *T. pictus*
- f) The kaplani group: *T. kaplani*, *T. pringlei*, *T. camdeboo*, *T. compassbergae*
- g) The black group: *T. stepheni*, *T. holmesi*, *T. brachycerus*, *T. tempe*, *T. yildizae*, *T. rileyi*, *T. petra*, *T. dukei*, *T. barbatus*, *T. penningtoni*

Table 3.1. Thestor: informal morphological groups based on wing coloration.

| Yellow | Black |
|-------------------------|-----------------------|
| <i>T. basutus</i> | <i>T. barbatus</i> |
| <i>T. braunsi</i> | <i>T. brachycerus</i> |
| <i>T. camdeboo</i> | <i>T. dukei</i> |
| <i>T. compassbergae</i> | <i>T. holmesi</i> |
| <i>T. dicksoni</i> | <i>T. penningtoni</i> |
| <i>T. dryburghi</i> | <i>T. petra</i> |
| <i>T. kaplani</i> | <i>T. rileyi</i> |
| <i>T. malagas</i> | <i>T. stepheni</i> |
| <i>T. montanus</i> | <i>T. tempe</i> |
| <i>T. murrayi</i> | <i>T. yildizae</i> |
| <i>T. kaplani</i> | |
| <i>T. pictus</i> | |
| <i>T. pringlei</i> | |
| <i>T. protumnus</i> | |
| <i>T. rossouwii</i> | |
| <i>T. swanepoeli</i> | |
| <i>T. strutti</i> | |
| <i>T. terblanchei</i> | |
| <i>T. vansonii</i> | |

The components of the genitalia studied by Alan Heath for possible variation are the uncus, aedeagus, valves, juxta, saccus and labides, the last of which is found only in the tribe Lachnocnemiini. The uncus is often (but not always) a character that is fairly stable within a genus. The general shape of the valves is also fairly stable within a genus but the apices are more often than not the character used for specific determination. The aedeagus is constant within a genus but small differences can occur. The juxta can either remain constant or can be used sometimes to distinguish species groups. The saccus is seldom used as a distinguishing character. Most species are determined by the valve apices (Heath personal communication). The genitalia of obligate ant-associated species vary little or not at all within a genus (Heath 1997 a; b) thereby necessitating the use of molecular characters to resolve relationships.

Phylogenetic analyses employing molecular data afford a powerful method of studying genera whose species show little morphological variation. Molecular phylogenetic estimates can be used to corroborate or question the basis of morphological placement of species, while providing insights into questions regarding taxonomy, biogeography, behavior and co-evolution. Mitochondrial DNA (mtDNA) is considered to be one of the best neutral markers to reveal phylogenetic relationships among related groups of insects, because mtDNA evolves fairly rapidly, most of the nucleotide substitutions occur at neutral sites, and mtDNA is unlikely to be responsible for morphological alterations (Brower 1994 a; Su et al. 1996 a; b). The determination of complete mtDNA sequences of the dipteran species *Drosophila yakuba*, coupled with the development of the polymerase chain reaction (PCR) have made it possible to amplify lepidopteran mitochondrial genes (which are compared to the corresponding sequences of *D. yakuba*) and to amplify their nucleotide sequences for phylogenetic analysis (Brower 1994 a; b; Campbell et al. 2000; Pashley et al. 1992; Rand et al. 2001; Taylor et al. 1993; Weller & Pashley 1995). In this study, we gathered data from regions of the mitochondrial genes Cytochrome Oxidase 1 (CO1 and subunit 5 of Nicotinamide Dinucleotide (ND5) to estimate relationships among species of *Thestor* and compare them with the hypothesis inherent in the morphological classification of Heath and Pringle (in preparation).

MATERIALS AND METHODS

Sampling

Samples were obtained for 29 morphologically designated species and subspecies of *Thestor*, representing almost the entire genus. *Thestor terblanchei*, *T. compassbergae*, and *T. dicksoni calviniae* could not be obtained. The majority of species were collected and identified by A. Heath as adults, and wings were removed and stored as vouchers. Specimens were stored immediately in the field in 100% ethanol, and later transferred to -80°C freezers in the DNA and Tissues collection of the Museum of Comparative Zoology Laboratories at Harvard University (Table 3.2). CO1 was sequenced for two or more individuals from each of the morphologically designated taxa, with the exception of

a few taxa in which only one individual was sequenced (see Table 3.2), resulting in a total of 65 CO1 sequences. ND5 was sequenced for one individual from 26 of the morphologically designated taxa. Owing to the fact that *Lachnocnema* was the only genus other than *Thestor* in the tribe Lachnocnemini (Eliot 1973), a species of *Lachnocnema*, *L. divergens*, was chosen as the outgroup.

Table 3.2. List of samples showing genes sequenced, range of distribution and altitude.

| Species | MCZ Accession No: | CO1 | ND5 | Range (> or < 20 sq. km) | Altitude |
|-----------------------------|-------------------|-----|-----|--------------------------|----------|
| <i>L. divergens</i> | RD-98-U130 | x | | > | M |
| <i>T. barbatus</i> | AH-00-T084 | x | | < | M |
| <i>T. basutus basutus</i> | AP-98-W770 | x | x | > | S |
| <i>T. taxon1</i> | AH-99-U475 | x | | < | S |
| <i>T. taxon5</i> | AH-98-Y796 | | | < | L |
| <i>T. taxon3</i> | AH-98 -Y816 | x | x | > | L |
| <i>T. braunsi</i> | DR-98-U633 | x | x | > | S/M |
| <i>T. braunsi</i> | AH-98-U642 | x | | > | S/M |
| <i>T. camdeboo</i> | AH-99-T280 | x | | < | M |
| <i>T. camdeboo</i> | AH-99-T281 | x | | < | M |
| <i>T. dicksoni dicksoni</i> | AH-00-T124/T125 | x | x | > | M |
| <i>T. dicksoni warreni</i> | AH-00-T158 | x | x | < | L |
| <i>T. dicksoni warreni</i> | AH-99-U528 | x | | < | L |
| <i>T. dryburghi</i> | AAM-98-V085 | x | | > | S |
| <i>T. dukei</i> | AH-98-U572 | | x | > | M |
| <i>T. dukei</i> | AH-98-U581 | | x | > | M |
| <i>T. dukei</i> | AH-98-U636 | | x | > | M |
| <i>T. dukei</i> | AH-98-U645 | x | x | > | M |
| <i>T. holmesi</i> | AH-00-T088 | x | x | > | M |
| <i>T. holmesi</i> | AH-99-U452 | x | | > | M |
| <i>T. holmesi</i> | AH-98-U625 | x | | > | M |
| <i>T. kaplani</i> | AH-99-U445 | x | x | < | M |
| <i>T. kaplani</i> | AH-98-U641 | x | | < | M |
| <i>T. malagas</i> | AH-00-T152 | x | | < | L |
| <i>T. malagas</i> | AH-00-T159 | x | x | < | L |
| <i>T. montanus</i> | AH-99-U429 | x | | > | M |
| <i>T. murrayi</i> | DR-98-U583 | x | | > | M |
| <i>T. murrayi</i> | AH-98-U592 | x | | > | M |
| <i>T. murrayi</i> | DR-98-U610 | x | x | > | M |
| <i>T. taxon2</i> | AH-00-T492 | x | x | < | L |
| <i>T. penningtoni</i> | AH-99-T274 | x | | > | M |
| <i>T. penningtoni</i> | NP-99-T454 | x | | > | M |
| <i>T. petra</i> | AH-99-U437 | | x | < | M |
| <i>T. petra</i> | AH-99-U438 | x | x | < | M |
| <i>T. pictus</i> | AH-99-U407 | x | | < | M |
| <i>T. pictus</i> | AH-99-U408 | x | x | < | M |

Table 3.2 (continued)

| Species | MCZ Accession No: | COI | ND5 | Range (> or < 20 sq. km) | Altitude |
|---------------------------------|-------------------|-----|-----|--------------------------|----------|
| <i>T. pringlei</i> | AH-99-U460 | x | | < | M |
| <i>T. pringlei</i> | AH-99-U462 | x | | < | M |
| <i>T. pro. protumnus</i> | AH-95-Y745 | x | | > | S/L |
| <i>T. pro. protumnus</i> | AH-95-Y752 | x | x | > | S/L |
| <i>T. pro. aridus</i> | AH-99-U420 | x | | > | S/L |
| <i>T. pro. aridus</i> | AH-99-U422 | x | x | > | S/L |
| <i>T. pro. mijburghi</i> | AAM-98-V080 | x | | < | L |
| <i>T. pro. mijburghi</i> | AH-98-Y444 | x | x | < | L |
| <i>T. rileyi</i> | AH-99-U451 | | x | > | M |
| <i>T. rileyi</i> | AH-99-U456 | x | x | > | M |
| <i>T. rooibergensis</i> | AH-99-U409 | x | x | < | M |
| <i>T. rooibergensis</i> | AH-99-U410 | x | | < | M |
| <i>T. rossouwii</i> | AH-98-Y702 | x | | > | L |
| <i>T. rossouwii</i> | AH-98-Y723 | x | | > | L |
| <i>T. taxon4</i> | AH-00-T082 | x | | < | M |
| <i>T. taxon4</i> | AH-00-T091 | x | x | < | M |
| <i>T. stepheni</i> | AH-00-T096 | x | x | > | M |
| <i>T. stepheni</i> | AH-99-U470 | x | | > | M |
| <i>T. strutti</i> | AH-99-U530 | x | x | < | M |
| <i>T. strutti</i> | AH-99-U545 | x | x | < | M |
| <i>T. swanepoeli</i> | AH-98-Y724 | x | | > | L |
| <i>T. swanepoeli</i> | AH-98-Y805 | x | | > | L |
| <i>T. sp. close penningtoni</i> | AH-00-T116 | x | x | < | M |
| <i>T. tempe</i> | AH-00-T312 | | x | < | M |
| <i>T. vansoni</i> | AH-99-U425 | x | | > | M |
| <i>T. vansoni</i> | AH-99-U426 | x | x | > | M |
| <i>T. yildizae</i> | DR-98-U570 | x | x | < | M |
| <i>T. yildizae</i> | AH-98-U599 | x | | < | M |

Note: in MCZ accession numbers: AAM – Andre Mignault, AH - Alan Heath, AP – Alan Plowes, DR – Douglas Rand, NP – Naomi Pierce, RD – Robert Ducarme. L = lowland (0-300m), S = submontane (300 – 1000 m), M = montane (> 1000m). *T. taxon 1*, *T. taxon 2*, *T. taxon 3*, *T. taxon 4* and *T. taxon 5* were formerly considered *T. brachycerus*, but A. Heath (personal communication) now considers their identification on the basis of morphology uncertain. *L. divergens* = *Lachnocnema divergens*, *T. basutus* = *Thestor basutus*.

DNA preparation, PCR and sequencing

To extract genomic DNA from preserved tissue, one third of the abdomen (two-three abdominal segments) was removed and homogenized in 2% SDS buffer, digested with Proteinase K and purified through two successive ethanol precipitations (100% and 70%). 998 bp of COI was amplified using two primer pairs, 'Ron/Nancy' and 'Tanya/Hobbes,' (see Rand et al. 2000) using the following PCR profile: 30-35 cycles of denaturing at 94°C for 45 seconds, annealing at 42°C for 60 seconds, and extension at 72°C for 90 seconds. 453 bp of ND5 was amplified by PCR using the primer pair V1 and C2 designed from the most conserved region of ND5 nucleotide sequences of *Drosophila melanogaster*, *D. yakuba*, *Carabus japonicus*, and *Anopheles gambiae* (Yagi et al. 1999). For ND5 the following PCR profile was used: 35-38 cycles of denaturation at 95°C for 60 seconds, annealing at 40°C for 60 seconds, and extension at 72°C for 90 seconds. All PCR products were purified with phenol/chloroform washes, gel separation, or Qiagen QiaQuick columns. PCR products were sequenced in both directions with Dye Terminator cycle sequencing using 'Ron/Nancy' and 'Tanya/Hobbes' for COI, and V1/C2 for ND5. Electrophoresis of sequenced products was carried out on ABI 377 and ABI 3100 (PE Biosystems) automated sequencers. Sequencher versions 3.0 (1995) and 4.1 (GeneCodes, Ann Arbor, MI) were used to align and edit all sequence fragments.

Biological data

Little biological information is available for the genus *Thestor*, with fewer than three partial life-histories described (Clark & Dickson 1971; Pringle et al. 1994; Heath & Claassens 2000). Hence, the evolution of characters for which information was available for all species was analyzed (Table 3.2). The geographic distribution and time of flight for the species of *Thestor* was also discussed. (Table 3.3).

Table 3.3. Life-history characteristics of *Thestor*.

| <i>Species</i> | <i>Heath's Species Groups</i> | <i>Distribution</i> | <i>Time of Flight</i> |
|-----------------------------------|---------------------------------------|--|---------------------------------------|
| <i>T. basutus basutus</i> | basutus | Eastern Cape Free State, KwaZulu Natal, Gauteng, Mpumalanga, Northern Province, Southern Botswana Lesotho, Zimbabwe | October - April |
| <i>T. basutus capeneri</i> | basutus | Gauteng | October - April |
| <i>T. protumnus aridus</i> | protumnus | Northern Cape, Free State | October - December |
| <i>T. protumnus mijburghi</i> | protumnus | Western Cape | September - December |
| <i>T. protumnus protumnus</i> | protumnus | Northern Cape, Eastern Cape, Western Cape | October - December |
| <i>T. dryburghi</i> | protumnus | Western Cape, restricted to Namaqualand | September - October |
| <i>T. rossouwi</i> | rossouwi | Southern Western Cape | October - April |
| <i>T. swanepoeli</i> | rossouwi | Southern Western Cape | November - January |
| <i>T. murrayi</i> | rossouwi | Western Cape | October - January |
| <i>T. strutti</i> | rossouwi | Western Cape | August - September |
| <i>T. braunsi</i> | dicksoni | Western Cape | October and March (double brooded) |
| <i>T. dicksoni dicksoni</i> | dicksoni | Western Cape | March - April |
| <i>T. dicksoni warreni</i> | dicksoni | Western Cape | March - April |
| <i>T. dicksoni calvaniae</i> | dicksoni | North Western Cape | December - February |
| <i>T. malagas</i> | dicksoni | Western Cape, restricted to Saldanha Bay | March |
| <i>T. montanus</i> | montanus | Western Cape | October - November |
| <i>T. vansoni</i> | montanus | Western Cape | October - November |
| <i>T. rooibergensis</i> | montanus | Southern Western Cape, restricted to the Rooiberg Mountains. | September - December |

Table 3.3 (continued)

| <i>Species</i> | <i>Heath's Species Groups</i> | <i>Distribution</i> | <i>Time of Flight</i> |
|-------------------------|---------------------------------------|---|------------------------|
| <i>T. pictus</i> | montanus | Southern Western Cape | November |
| <i>T. compassbergae</i> | kaplani | Western Cape | December |
| <i>T. kaplani</i> | kaplani | Southern Western Cape | December – January |
| <i>T. pringlei</i> | kaplani | Western, North-western Cape | December |
| <i>T. camdeboo</i> | kaplani | Eastern Cape | November – December |
| <i>T. stepheni</i> | black | Western Cape | December – January |
| <i>T. holmesii</i> | black | Western Cape | December – January |
| <i>T. brachycerus</i> | black | Western Cape | October - February |
| <i>T. tempe</i> | black | Western Cape | October – January |
| <i>T. yildizae</i> | black | Western Cape (restricted to Table Mountain range) | November - February |
| <i>T. rileyi</i> | black | Southern Western Cape | December – January |
| <i>T. petra</i> | black | Western Cape | November – January |
| <i>T. dukei</i> | black | Southern-Western Cape | November – January |
| <i>T. penningtoni</i> | black | Western Cape | October – November |
| <i>T. barbatus</i> | black | Western Cape | December – January |

Phylogenetic analyses

Phylogenetic analyses employed parsimony and Bayesian likelihood optimality criteria. A total of 1393 bp were used for phylogenetic analyses, 998 from CO1 and 395 from ND5. To explore the possibility of saturation in the data, scatter plots of transition/transversion ratios versus total distance were constructed for pairwise comparisons in each gene region (Figures 3.1a and 3.1b). The presence of conflicts in phylogenetic signal between the CO1 and ND5 data sets was tested using the

incongruence length difference (ILD) test (Farris et al. 1995), implemented as the partition homogeneity test in PAUP* (Swofford 1998) under a heuristic search with 100 replications. The two data sets (CO1 and ND5) were analysed separately as well in combination.

Parsimony analyses

Separate and combined analyses of the two data sets were performed with all characters equally weighted, with transitions down-weighted by a half and by a third in third codon positions, as well as in all codon positions. Parsimony analyses were performed in PAUP* 4.0b1a for Macintosh (Swofford 1998), employing a heuristic search with 1000 random addition sequence (RAS) replicates and tree-bisection-reconnection (TBR) branch swapping. Clade support was assessed from 100 bootstrap replicates using 100 random addition replicates in PAUP*. The program TreeRot 2.0 (Sorenson 1999) was used to create the constraint trees for the calculation of the partitioned Bremer support. Decay indices were calculated from these constraint trees from runs performed in PAUP* using heuristic searches with 100 random-addition starting trees. MacClade version 3 (Maddison & Maddison 1992) was used to visualize trees for character optimization.

Bayesian Likelihood analyses

For both separate and combined data sets, Modeltest (Posada 1998) was used to select the best-fitting model for likelihood analyses. Modeltest implements hierarchical likelihood ratio tests (LRT) of significant differences between increasingly complex substitution models to select the best fitting model with the minimum number of parameters. The selected model for all three data sets (separate and combined) was a General Time Reversible (Rodriguez et al. 1990) model, estimating the proportion of invariable sites and the shape of the gamma parameter (GTR+I+G). This model was then incorporated into Bayesian phylogenetic searches using MrBayes 2.01 (Huelsenbeck & Ronquist 2001). Bayesian searches were conducted using four simultaneous chains run for 2 million generations and sampled every 100 generations. Two such searches were

carried out at a temperature of 0.5 and 0.3, thereby implementing different switching rates between chains. We also conducted a site-specific search in MrBayes with among-site variation where rates within a class varied according to a gamma distribution. Trees with pre-asymptotic likelihood scores were discarded and the remaining trees were used to compute the average-branch-length consensus tree, the majority rule consensus topology and posterior probabilities.

Testing morphologically based hypotheses

The monophyly of some of the morphological groupings of Heath and Pringle (personal communication) was tested in cases where they were not found in any of the trees obtained from the molecular phylogenetic analyses. For this, Templeton's Wilcoxon signed rank test (Templeton 1983) and Prager and Wilson's (1988) winning sites test, both implemented in PAUP*, were used to examine whether or not the monophyly of these groups significantly contradicted the molecular data (Table 3.3). In these tests, the branch lengths of the constrained topologies are compared to the topologies obtained by parsimony analyses of the molecular data, and then tested for significant differences. The morphological groups were separately constrained to be monophyletic and parsimony searches using the same parameters as in the initial search were performed.

RESULTS

Of the 998 characters in the 65-taxon CO1 data matrix, 457 (45.79%) were variable and 326 (32.66%) are informative. The informative characters tended to occur far more frequently in the more unconstrained third positions than in first or second positions (Figure 3.1). The sequences possessed a considerable A-T bias (71%) over C-G (29%).

In similar fashion, the 26 taxon ND5 data matrix had 115 variable (29.11%) and 81 informative (20.50%) sites out of a total of 395 characters (Figure 3.2). Again, these sequences possessed an AT bias, accounting for 79.37% of the bases.

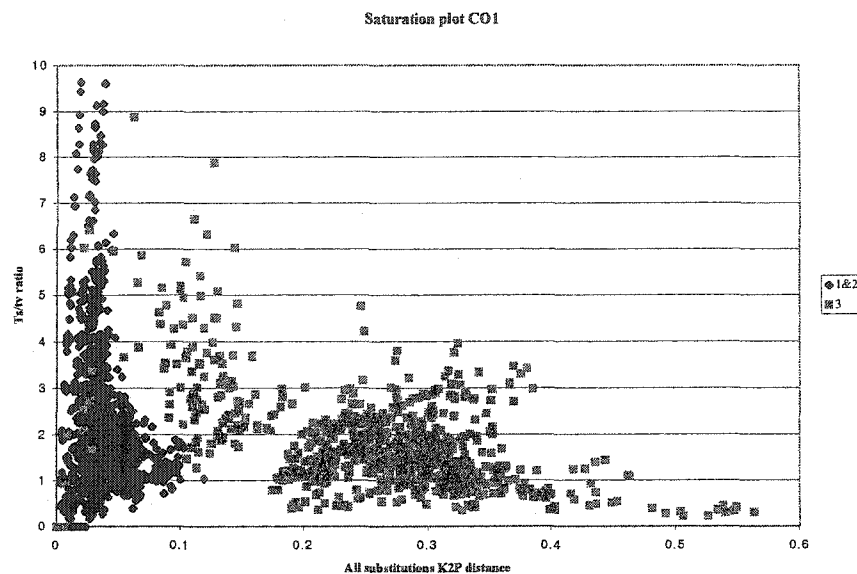


Figure 3.1a. Transition/transversion ratio vs. Total number of pairwise differences for CO1.

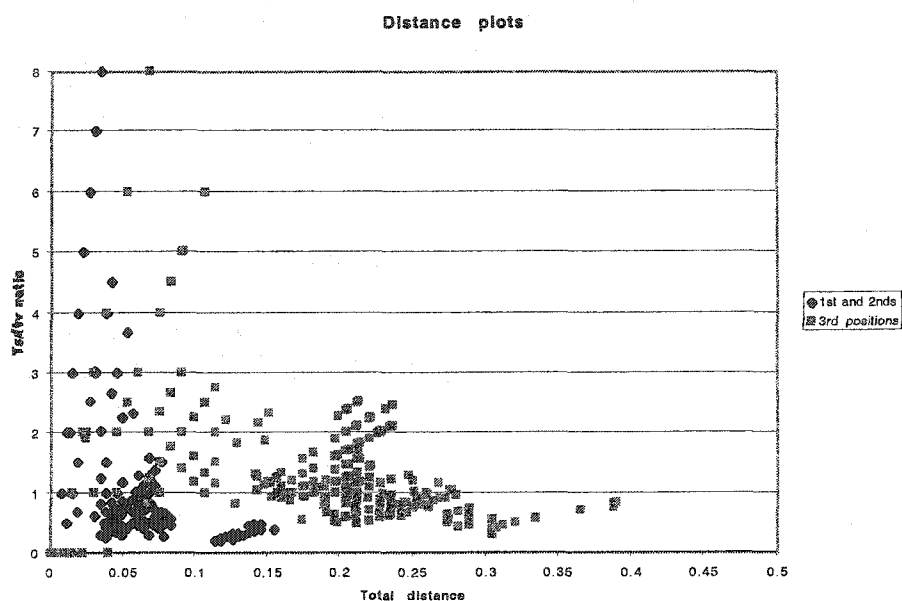


Figure 3.1b. Transition/transversion ratio vs. Total number of pairwise differences for ND5.

Heuristic searches, with random taxon addition, yielded nine most parsimonious trees for CO1 with flatweighting (Figure 2.2). There was no change in the topology or in the resolution when down-weighting 3rd positions of all substitutions equally. The length of

the tree was 1310 with flatweighting. The consistency index (CI) was 0.357 and the retention index (RI) was 0.82.

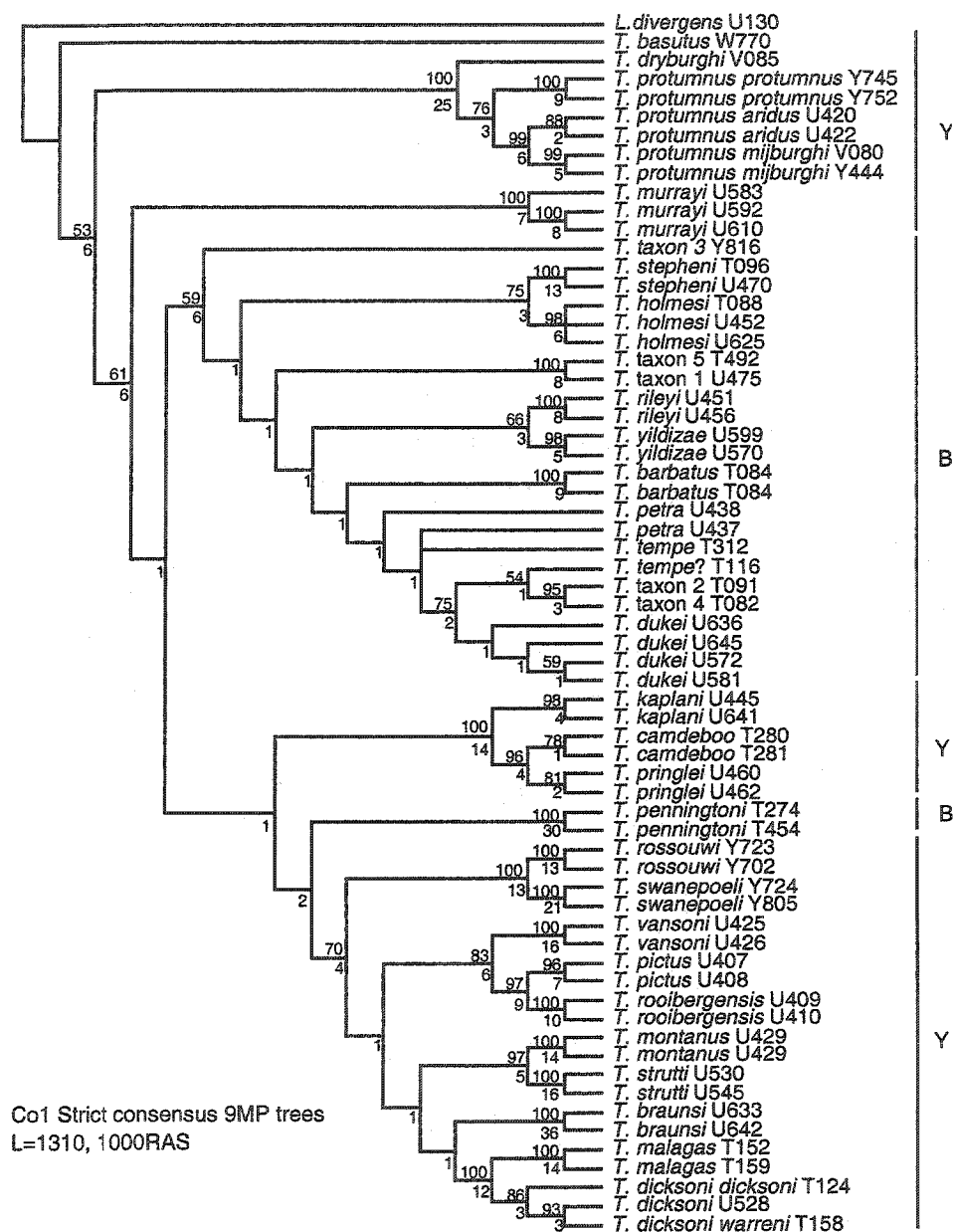


Figure 3.2. Strict consensus CO1 with bootstrap values and decay indices.

With the puzzling exception of *T. penningtoni*, the black species of *Thestor* appeared to form a monophyletic group. The yellow *Thestor*, however, were paraphyletic and did not support a clean morphological demarcation on the basis of color alone.

Under a strict parsimony consensus condition using CO1, Heath's informal species groupings were maintained for the following groups. The decay indices and bootstrap consensus for these groups offered independent perspectives on clade robustness.

- a) The protumnus group, including *T. protumnus protumnus*, *T. protumnus aridus*, *T. protumnus mijburghi* and *T. dryburghi*. This grouping was very well supported with a bootstrap value of 100% for the clade and the decay index is 25.
- b) The dicksoni group, including *T. dicksoni*, *T. malagas*, and *T. braunsi*. The malagas-dicksoni grouping was particularly well supported with a bootstrap value of 100% and a decay index of 12.
- c) The kaplani group, including *T. kaplani*, *T. camdeboo*, and *T. pringlei*. There was very high bootstrap support for this clade (100%), and a high decay index (14). The fourth species in this morphological grouping, *T. compassbergae*, could not be obtained for sampling.
- d) All the black *Thestor* species, with the exception of *T. penningtoni*. The bootstrap support was not very high, however (59), and the decay index was 6.

The CO1 most parsimonious (MP) tree (Figure 3.2) supported Heath's informal hypothesis, based upon his genitalic studies, that *T. basutus* is the most basal taxon, followed closely by the protumnus clade.

Morphological groupings are optimized on the topology as shown in Figure 3.3.

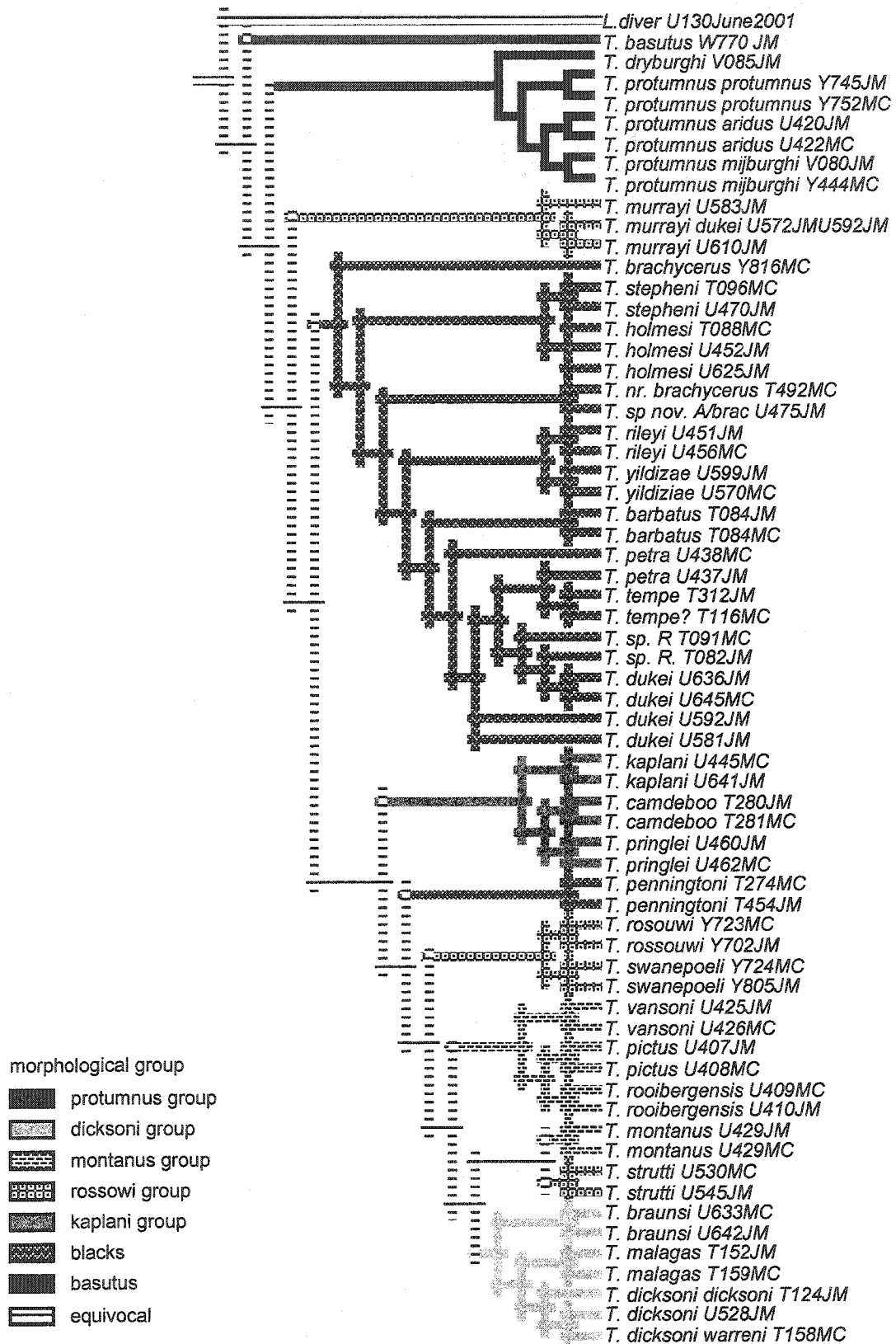


Figure 3.3 Optimization of morphological groupings on COI MP tree.

In addition, some of the species included in the other informal morphological groupings, emerged together (Figure 3.2, 3.3).

- a) For the rossouwi group, *T. rossouwi* and *T. swanepoeli* were each other's closest relatives. The bootstrap support was very high (100%) as was the decay index (13). However, *T. murrayi* and *T. strutti* emerged separately.
- b) For the montanus group, *T. pictus*, *T. vansonii* and *T. rooibergensis* were placed together, with a high bootstrap support of 83 and a decay index of 6. However, *T. montanus* itself emerged elsewhere on the tree, 2 nodes away, with its closest relative appearing to be *T. strutti*.

Alternative topologies for Heath's groupings that were not supported (in whole or in part) by the molecular data were evaluated and results shown in Table 3.4.

Table 3.4. Testing morphological groups (A. Heath) under parsimony (CO1).

| Group Constrained | Number of trees | Length difference | Parsimony | | Winning sites | Likelihood S-H |
|-----------------------------------|--------------------|----------------------|------------------------|------------------------|----------------------|-------------------|
| | | | K-H | T | | |
| Montanus group | 36 | 10 | 0.0864- 0.0123*(12) | 0.0886- 0.0075*(12) | 0.136- 0.0129*(9) | 0.229- 0.108 |
| Rossouwi group | 90 | 27 | 0.0034*- 0.0015* | 0.0036*- 0.0014* | 0.0142*- 0.003* | 0.009*- 0.001* |
| Black group (sensu Heath) | 35 | 41 | <0.0001* | <0.0001* | <0.0001* | <0.0001* |
| Black group polytomy | 10 | 31 | 0.0014*- 0.0032* | 0.0016*- 0.0046* | 0.0058*- 0.0146* | <0.0001* |
| Yellow and black (as Bayesian) | 13 | 61 | <0.0001* | <0.0001* | <0.0001* | <0.0001* |
| Yellow group | 54 | 38 | 0.0087*- 0.0069* | 0.0107*- 0.0072* | 0.0269*- 0.0203* | 0.002*- 0.001* |

(K-H – Kishino-Hasegawa, T – Templeton, S-H – Shimodaira-Hasegawa. Range values refer to p values obtained under parsimony for each of the constraints. Asterisks refer to significant values. Bracketed numbers refer to the number of trees that show significant differences of length).

Only the montanus group showed non-significant p values included in the range, which suggests the grouping is not significantly rejected for most trees. This could be accounted for by the fact that *T. montanus* was separated from the rest of the morphological 'montanus' grouping (*T. pictus*, *T. vansonii* and *T. rooibergensis*) by only 2 nodes.

In the Bayesian Likelihood tree (Figure 3.4), a slightly different scenario emerged. The black *Thestor* were supported by a posterior probability of 95%, with *T. penningtoni* emerging as part of the group, though basal to all other black *Thestor* species, which have posterior probability support of 99%. *Thestor braunsi* did not emerge with *T. dicksoni* and *T. malagas*, unlike in the MP tree. The montanus morphological grouping (with the exception of *T. montanus*, which also emerged separate from the other species in that morphological grouping for the COI MP tree) was placed most closely to the protumnus grouping, essentially switching positions with *T. murrayi*, when compared with the COI MP tree. With these exceptions, the MP tree and Bayesian Likelihood tree had much in common. The montanus grouping minus *T. montanus* (*T. kaplani*, *T. camdeboo* and *T. pringlei*) had posterior probability support of 98%, the dicksoni grouping (*T. dicksoni dicksoni*, *T. dicksoni warreni*, and *T. malagas*) of 100%, and the vansoni grouping (*T. vansoni*, *T. pictus*, and *T. rooibergensis*) of 99%. *Thestor rossouwi* and *T. swanepoeli* also emerged together with posterior probability support of 99%. The given relationships all supported Heath and Pringle's (informal) groupings. Significant divergences from Heath and Pringle's groupings were seen in the following:

- a) the placement of *T. montanus* separate from the other species included in the montanus morphological grouping.
- b) the grouping of *T. montanus* with *T. strutti* (this grouping had a strong posterior probability of 100, which, coupled with 97% support for the same grouping in the COI MP tree suggests that the relationship may be real).
- c) the placement of *T. braunsi* relative to the dicksoni group.

Majority rule

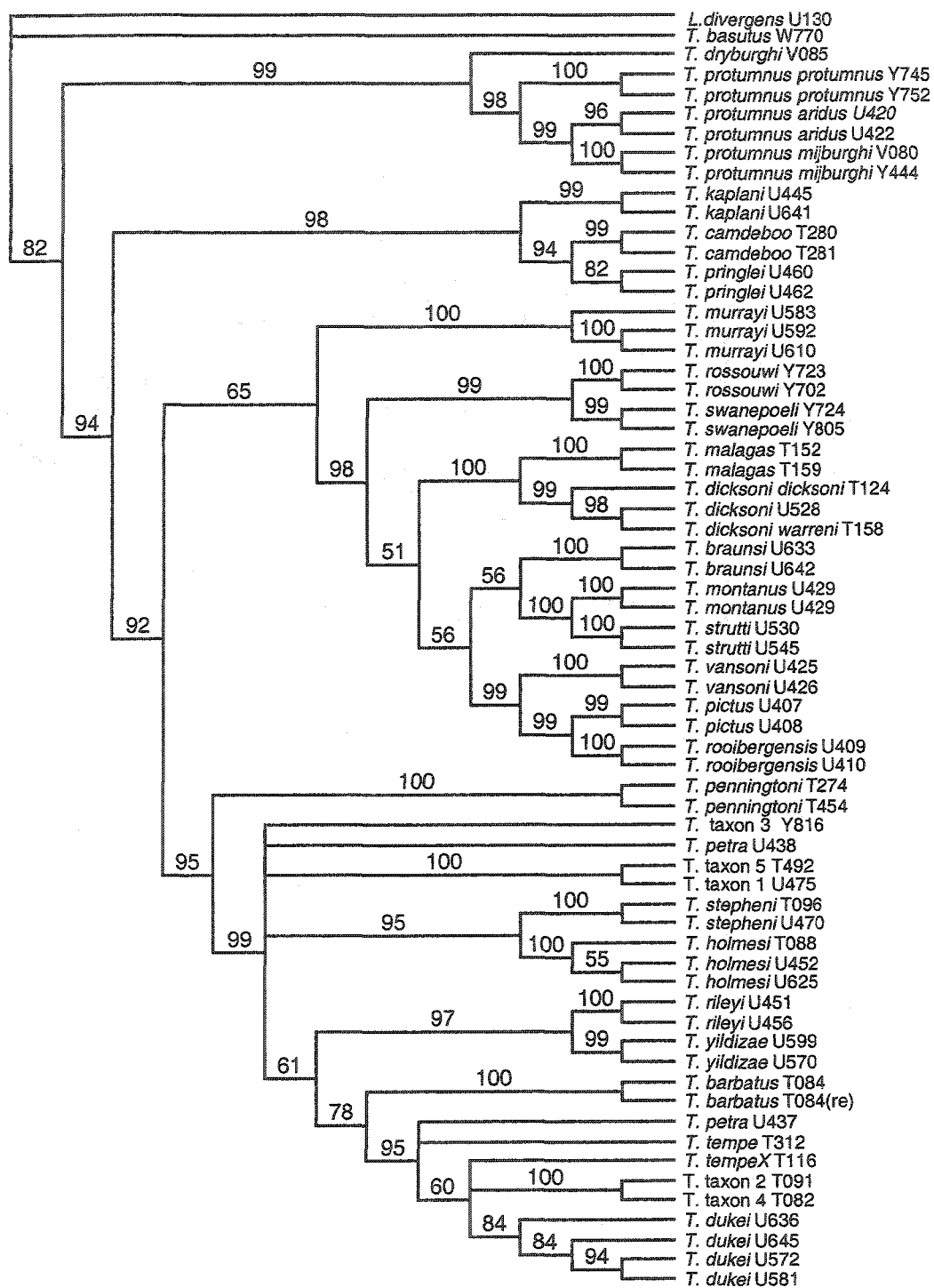


Figure 3.4. Bayesian topology, Majority Rule for CO1.

A different scenario emerged from an analysis of ND5 data (Figure 3.5). Here, *T. penningtoni* was recalcitrant to amplification and, hence, could not be included in the analysis, which rendered the unusual position of this taxon in the phylogeny unconfirmed in the ND5 MP tree. In the ND5 MP tree, the black *Thestor* species emerged as monophyletic, while the yellow *Thestor* were paraphyletic. Particularly intriguing was the placement of *T. kaplani* closest to the black *Thestor* species. However, the fact that two species found in the montanus group that emerged closest with *T. kaplani* in the CO1 MP tree, *T. pringlei* and *T. camdeboo* could not be amplified for ND5 might have affected the final placement of *T. kaplani* on the tree. The protumnus grouping was maintained with 100% support; however, *T. dryburghi* could not be amplified for ND5. The grouping of *T. pictus* and *T. rooibergensis* was intact with a bootstrap value of 92; however, *T. vansonii* emerged paraphyletic to this grouping (though the support here was less than 50%, and so is not reflected in the tree). The dicksoni group, with bootstrap support of 65% included all of Heath and Pringle's placements (*T. braunsi*, *T. dicksoni warreni*, *T. dicksoni dicksoni* and *T. malagas*). The additional placement of *T. basutus* in this clade, was particularly anomalous, because in Heath and Pringle's groupings, as well as in the CO1 MP and Bayesian trees, *T. basutus* was the most basal taxon, whereas in the ND5 MP tree, it was the most derived. Furthermore, *T. basutus* was placed most closely with *T. malagas* in the ND5 tree, with 96% bootstrap support, which is in considerable conflict with the suggested morphological groupings and the CO1 data mentioned. *Thestor braunsi* emerged again separate from *T. dicksoni* and *T. malagas*.

All differences and agreements in topology between CO1 and ND5 MP trees are summarized in figure 3.6. Node 1 shows the monophyly of the protumnus group, Node 2 the *T. pictus*-*T. rooibergensis* clade, Node 3, the monophyly of the black *Thestor* (with the exception of *T. penningtoni*, that was not included in the analysis), and Nodes 4, 5 and 6 refer to internal structure within the black *Thestor* clade.

A Bayesian Likelihood search using ND5 resulted in a topology similar to the ND5 MP tree (with the exception that the black *Thestor* species are not a single monophyletic clade, but two), and is not shown here. Like in the ND5 MP tree, *T. basutus* again emerged as derived, with *T. malagas*, with posterior probability support of 100%. Such a

placement of *T. basutus* is in conflict based on all other evidence from morphology and CO1, where *T. basutus* consistently emerges basal to all other *Thestor* species.

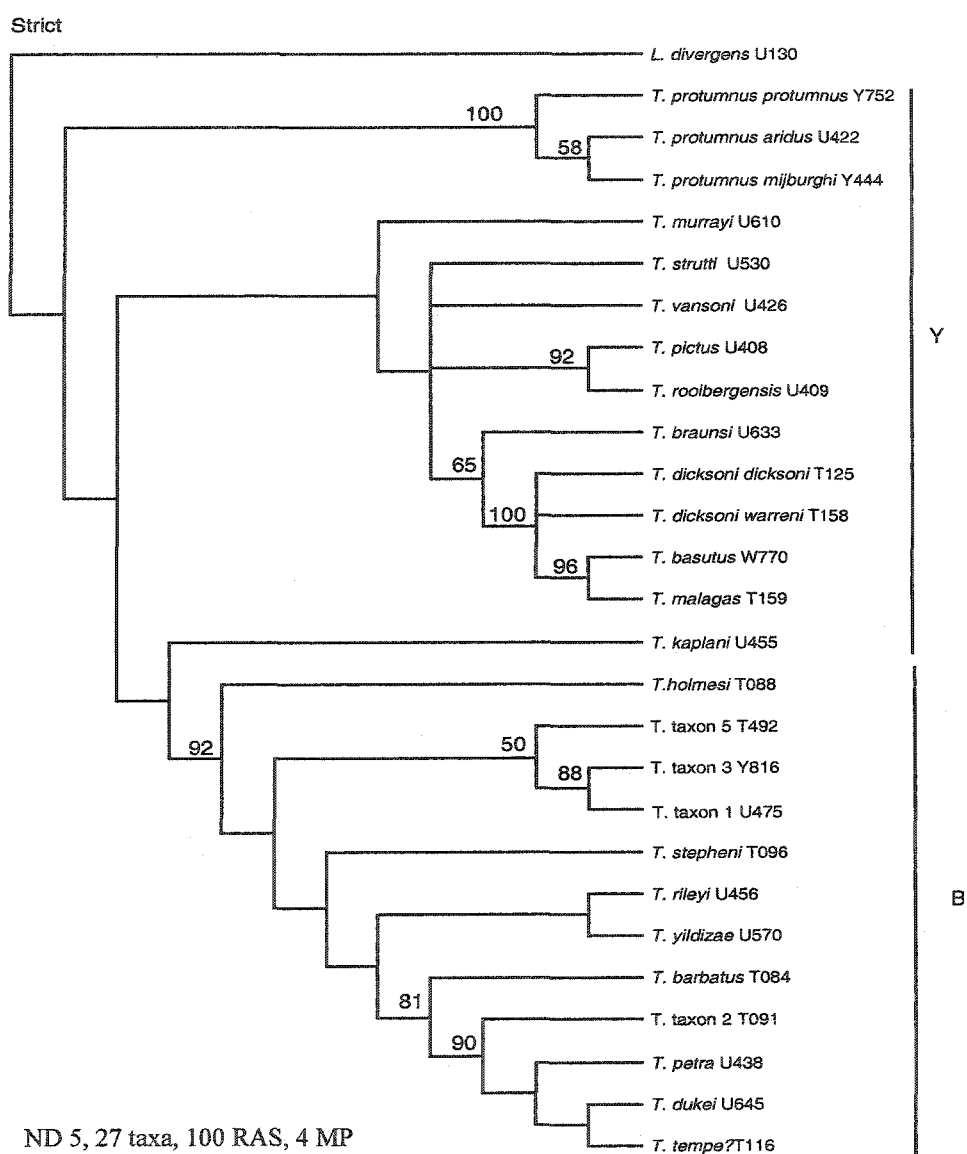
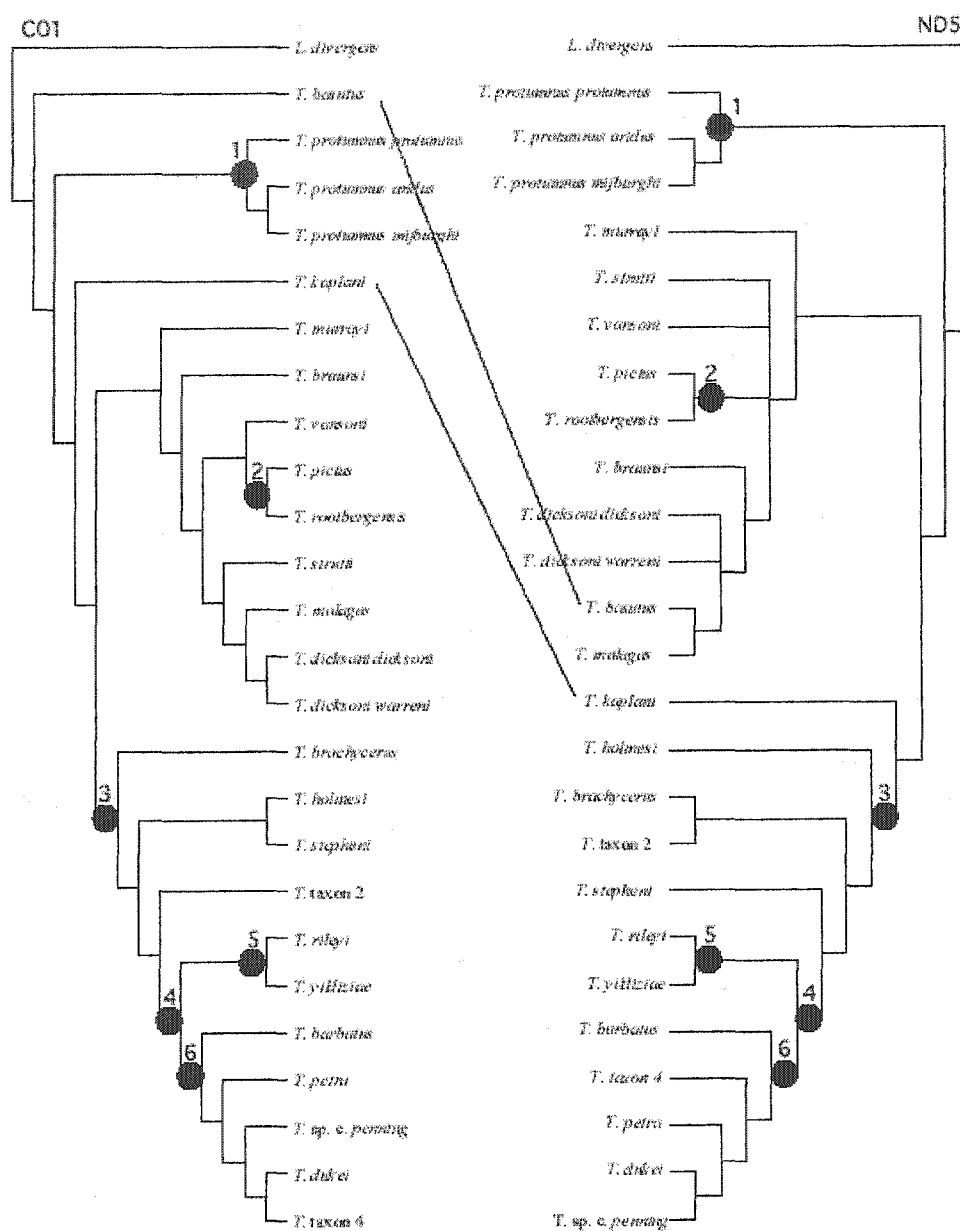


Figure 3.5. Strict consensus with bootstrap values for ND5.



ILD P value = $1 - (99/100) = 0.010000$

Figure 3.6. Comparison of CO1 and ND5 topologies, with ILD value.

When the two genes were analyzed together (Figure 3.7), the conflicting positions of *T. kaplani* and *T. basutus* resulted in several polytomies in the strict consensus tree. The partition homogeneity tests yielded a significant ILD p value of 0.01 (Figure 3.6).

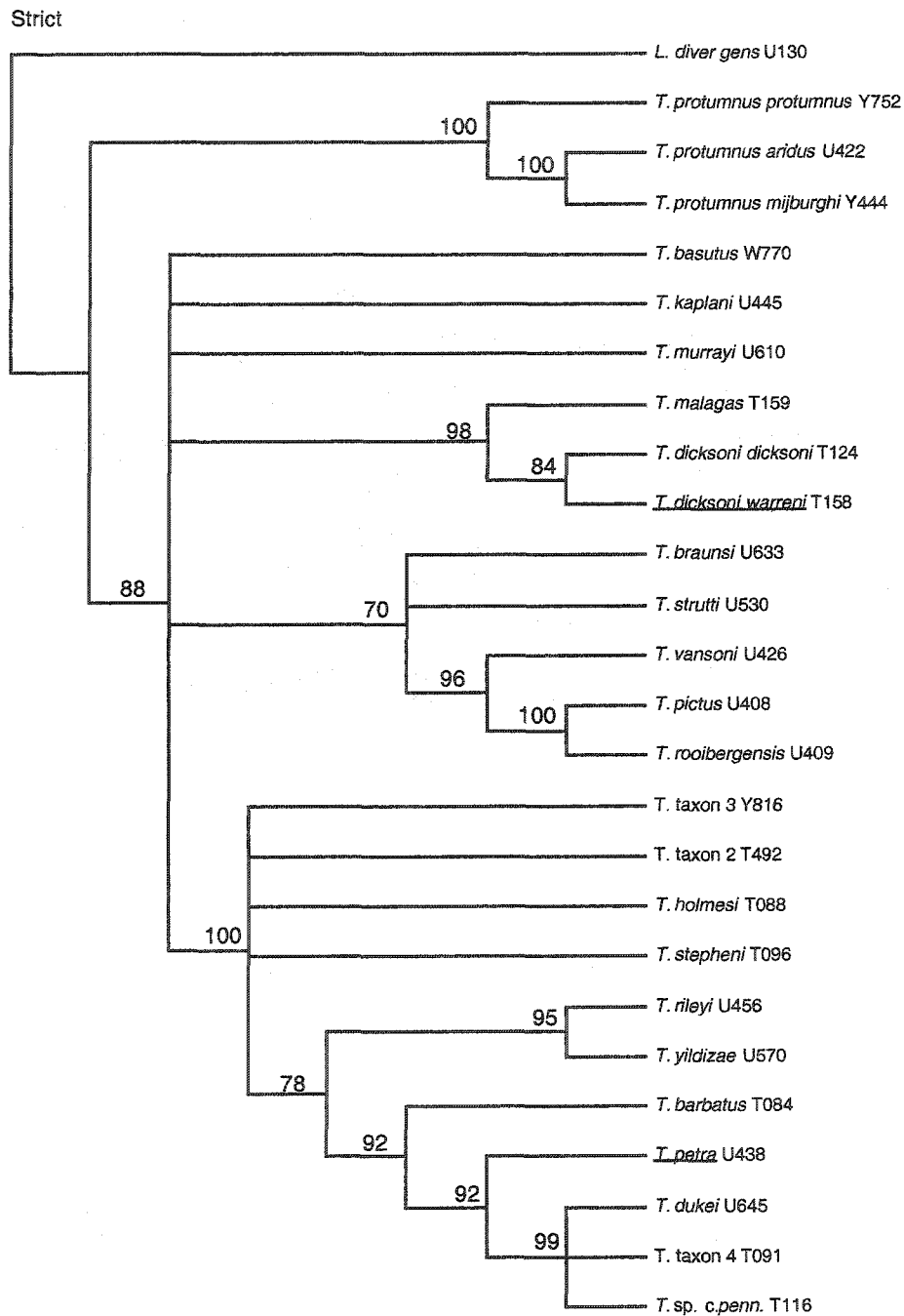


Figure 3.7. Strict Consensus COI-ND5 MP Tree.

DISCUSSION

For purposes of discussing natural history characteristics with respect to the phylogeny, the CO1 MP tree (Figure 3.2) has been employed, since it appears to give the most reliable estimates in relation to a priori morphological placements of taxa within *Thestor*, apart from possessing the greatest number of sampled taxa.

Phylogeny

On the basis of the CO1 topology, using both the CO1 MP tree with supporting bootstrap values and the Bayesian tree with supporting posterior probabilities, it is possible to evaluate the morphological basis for delineation of groupings (Table 3.3). The yellow *Thestor* are not monophyletic; however, the black *Thestor* appear to be. The one possible exception is *Thestor penningtoni*, which is found separate from the other black *Thestor* species in the CO1 MP tree and among the yellow *Thestor*, though along with the black *Thestor* in the Bayesian tree. The unfortunate lack of success with amplifying *T. penningtoni* for the gene ND5 leaves the problem unresolved, though it has been pointed out earlier that ND5 may not be the best gene of choice for the phylogenetic analysis of this genus. This fact is particularly relevant in determining the status of *T. brachycerus*, since Heath (personal communication) has revised his opinion concerning his identification of several specimens that he originally believed were populations of the genus. He now believes he cannot identify them positively and so prefers to consider them *T. taxon 1*, *T. taxon 2*, *T. taxon 3*, *T. taxon 4* and *T. taxon 5*.

Both bootstrap values and posterior probabilities for the protumnus group, the dicksoni group and the kaplani group are exceedingly high (100 % for bootstraps in all cases, and above 98 for the posterior probabilities) (Figures 3.2, 3.4), which support the predictions of Heath and Pringle (unpublished). For the dicksoni group, Heath (personal communication) maintains that *T. malagas* is only a variant of *T. dicksoni* and so may merit no more than subspecific status. He advances the same claim in the protumnus morphological grouping for *T. dryburghi*, stating that it could well be a subspecies of *T. protumnus*, so also *T. terblanchei*, which, unfortunately, could not be included in the molecular sampling strategy owing to lack of availability.

In the case of the black *Thestor* species, the morphological predictions of Heath and Pringle (personal communication) are also upheld, although with lower bootstrap support (59%) in the CO1 MP tree, though with Bayesian posterior probability support of 95. The black *Thestor* species form an intriguing problem for the morphologists - Heath (personal communication) maintains that the variation in genitalia is not pronounced and he is often inclined to consider all of the black species one large species with subtle variations. The molecular data certainly support a monophyletic clade (with the exception of *T. penningtoni* in the CO1 MP tree), with some internal resolution, however, including a grouping of *T. rileyi* and *T. yildizae* (66% bootstrap support) and *T. stepheni* and *T. holmesi* (75% bootstrap support), which does indicate some phylogenetic structure.

The basutus group, which contains two subspecies of *T. basutus*, is represented in the molecular phylogeny by just one specimen (*T. basutus basutus*) owing again to lack of availability, and hence its monophyly cannot conclusively be established. The CO1 trees support the basal placement of the taxon as proposed by the morphological data, as well as close to basal placement of the protumnus clade. It is particularly interesting that *T. basutus* is completely allopatric to all other *Thestor* species, and is one of the few taxa in the genus not to be found in the Western Cape. If *T. basutus* is truly basal, the suggestion may be advanced that *Thestor* originated in the more northern provinces of South Africa and then expanded its range, with the most successful radiation occurring in the Western Cape. This assertion may have some backing, in that the species included in next most basal group to *T. basutus*, the protumnus clade (*T. protumnus protumnus*, *T. protumnus aridus*, *T. protumnus mijburghi* and *T. dryburghi*), have ranges that are distinctly more northern than the species found in the more derived groups, which are largely restricted to the Eastern and Western Cape provinces, and the southern portions of the Northern Cape. All of the species of one of the most derived clades, the black *Thestor* are completely restricted to the Western Cape.

The rossouwi group contains two of the four species originally attributed to it, namely *T. rossouwi* and *T. swanepoeli*, which have 100% bootstrap support and a posterior probability of 99%. Heath (personal communication) contends that *T. rossouwi* and *T. swanepoeli* are in all likelihood the same species and advocates sinking them into one species. *Thestor murrayi* and *T. strutti*, however, emerge separately on the tree and their

placements do not support the morphological hypothesis. It is interesting to note that the placement of *T. murrayi* is substantially distant from the *T. rossouwi* – *T. swanepoeli* clade in the CO1 MP tree, though only one node apart in the CO1 Bayesian tree.

In the montanus group, the only species that fails in the molecular analysis to find inclusion is *T. montanus* itself. The other species, *T. vansonii*, *T. rooibergensis* and *T. pictus*, all emerge closest to each other in both MP and Bayesian trees for CO1, with values of 83% bootstrap and 99% posterior probability, respectively. *T. rooibergensis* and *T. pictus* are particularly closely placed, with a bootstrap value of 97% and posterior probability of 99%, although Heath has not recommended that they be collapsed into a single species. This may be because the ranges of the species do not overlap and the potential for further divergence through isolation (if not already in place) is likely.

Life-history characteristics

One of the chief questions that attend the remarkable radiation of the genus *Thestor* into so many morphological species and subspecies is what factors may have contributed to such release. The lack of substantial life-history data on the genus leaves few pointers to suggest correlations. The feeding patterns of only three species of this aphytophagous genus are known – *T. protumnus aridus* has been seen to feed on homopterans in the early instars (Clark & Dickson 1960), *T. basutus* feeds on detritus (Heath & Claassens 2000), by trophallaxis with adult ants and occasionally on ant brood (Heath, personal communication) and *T. yildizae* feeds by trophallaxis with adult ants (Heath & Claassens 2000). The great anomaly that characterizes the extensive radiation of the genus is that in all circumstances, larvae appear to be attended by only one species of ant, the formicine *Anoplolepis custodiens*. Quek (unpublished) has collected several colonies of ants tending different species of *Thestor* and subjected them to phylogenetic analysis to ascertain whether what purports to be one species of ant is in reality a suite of cryptic species. Her initial results suggest that there are indeed differences among populations sampled, but these do not correspond readily with differences in species of *Thestor*, suggesting that perhaps a co-speciation scenario is not in evidence. If anything, the

butterflies do not appear to be driving speciation in the ants, though it is not clear whether the reverse may be true.

An important point to be examined is whether the range of each species of *Thestor* was a function of phylogenetic constraint, and whether such restriction is attendant upon the altitude at which particular species are typically found. Information presented in Table 3.2 does not reflect such a trend, and it is evident that sister taxa (such as *T. dicksoni dicksoni* and *T. malagas*) (Figure 2.2) can show both marked altitudinal differences in location, as well as in magnitude of range. Furthermore, seeing as *T. malagas* is confined to a small stretch of beach habitat, while *T. dicksoni dicksoni* is montane and widespread, the argument for specialized exploitation of habitats driving diversification and even speciation may also be advanced.

Time of flight shows some interesting patterns (Table 3.2) when considered with respect to molecular groupings in comparison to morphological groups. In the basutus group, *T. basutus* has the longest flight time of October to April. Conceivably, this may tie in to the fact that the species is completely allopatric to all other species, which suggests that there is no immediate niche partitioning requirements with congeners; although *T. rossouwi* has a comparable time of flight, it is not sympatric with *T. basutus*. In the protumnus group, the flight occurs between September and December. The species do not overlap much spatially, so temporal coincidence is unlikely to be an issue. In the reduced rossouwi group (*T. rossouwi* and *T. swanepoeli*, as supported by the molecular analysis), *T. rossouwi* flies between October and April and *T. swanepoeli* between November and January. The morphological rossouwi grouping also includes *T. murrayi* and *T. strutti*. *Thestor murrayi* almost completely overlaps *T. swanepoeli* temporally though not spatially, and *T. strutti* flies only between August and September, which may suggest niche separation, since both *T. murrayi* and *T. strutti* are restricted to the Western Cape. In the dicksoni clade, *T. braunsi* is the only species of *Thestor* that is known to be double brooded (Pringle et al. 1994), occurring in both October and March. All other species in the group fly between March and April with the exception of the North-Western Cape restricted *T. dicksoni calviniae* (not available for inclusion in the phylogenetic analysis), which flies between December and February. Since all other species are found in the Western Cape with *T. malagas* restricted to Saldanha Bay, it is

intriguing that *T. dicksoni calviniae* is both temporally and spatially separated. Other unsuspected ecological constraints might be involved. In the montanus morphological grouping, the window for flight-times is between October and December. The greatest restriction appears to be found in the kaplani group where *T. camdeboo* flies only in November and December, *T. kaplani* flies in December and January, *T. pringlei* only in December, and *T. compassbergae* also only in December, apparently for no more than a week each year (Heath personal communication). *Thestor compassbergae* is particularly enigmatic – it is found in the remote Compassberg mountains and its ephemeral appearance remains inexplicable, particularly when no competition appears immediately evident for resources. Among the black *Thestor*, most species are found flying between October and January, with only *T. brachycerus* (which may be represented by more than one species), and *T. yildizae* extending into February.

CONCLUSIONS

The morphological basis of dividing *Thestor* on the basis of color into yellow and black *Thestor* is partially flawed in that the yellow *Thestor* are not a monophyletic group separate from the monophyletic black *Thestor*. Rather, they are paraphyletic, because they include the monophyletic black *Thestor* clade. The informal groupings of Heath and Pringle (personal communication), however, are largely valid, with only a few exceptions, particularly in the rossouwi and montanus groups (and in the latter case, only as regards a single species), the vexing case of *T. penningtoni* in relation to the other black *Thestor* species, and the position of *T. basutus* in the phylogeny, be it either basal or derived. Ant association appears to be obligate, and there is the suggestion that the hitherto sole suspected species of ant attending all the species of *Thestor* may actually be a suite of cryptic species; however, there is little evidence as yet to suggest that co-speciation is occurring. Range and altitude for individual species do not appear to be immediately constrained phylogenetically. Time of flight may be governed to some extent by pressures of niche partitioning. It may be that specialization of habitats could have driven the diversification of species, and that non-overlapping ranges, particularly

with respect to the montane taxa, might have aided the radiation into so many species as are found included in the genus *Thestor*.

SECTION 4

THE SINGING REAPER: DIET, MORPHOLOGY AND VIBRATIONAL
SIGNALLING IN *FENISECA TARQUINIUS* (LYCAENIDAE, MILETINAE)

Life-history

The life-history of the only known carnivorous North American butterfly, *Feniseca tarquinius* (Lycaenidae: Miletinae), the harvester, has been known for over a century (Edwards 1886; Riley 1886; Scudder 1889; 1897), yet little has appeared recently on the ecology of this enigmatic species. *F. tarquinius* is encountered sporadically across its range, from Florida in the south to Nova Scotia in the north, and as far west as Texas in the south and Manitoba in the north (Opler & Krizek 1984). Up to eight broods have been reported for *F. tarquinius* in Virginia (Clark & Clark 1951). More typically, however, it is known to have two to three broods, the first appearing in early May, the second in mid to late July, and occasionally, a third in August or September. The butterfly lays its eggs among clumps of woolly aphids (Aphidoidea: Pemphigidae) (Figure 4.1), on which the emergent larvae obligately feed (Scott 1986) (Table 4.1). The homopteran prey is typically attended by ants which have been observed on occasion to be distinctly hostile to the lycaenid larvae (Scudder 1889). This possibly explains why young instar larvae tend to live in concealed locations under the aphids until fully grown (Figure 4.2), even spinning a silken web among the aphids (Clark 1926; Cottrell 1984). As frequently noted in the literature, the shape and markings on the pupa render it in appearance similar to a miniature monkey's head (Scudder 1897; Balduf 1939; Hinton 1974; Krizek 1995) in which it is resembled by some other members of the tribe Spalgini (Lycaenidae: Miletinae) to which *F. tarquinius* belongs. Development appears to be relatively rapid, lasting approximately three weeks from egg to adult (Scott 1986).

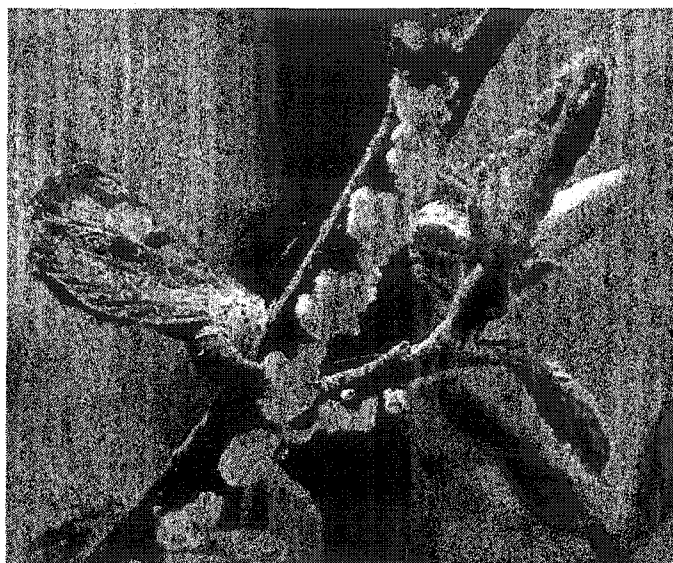


Figure 4.1. Female ovipositing on underside of branch into host aphid colony (Photograph by David Small).

Table 4.1. Aphid prey of *Fenisea tarquinius* and their reported host-plants.

| No: | Associated Aphids* | Host plant species* | Common Name | Family |
|-----|--------------------------------------|-----------------------------|--------------------|----------------|
| 1 | <i>Neoprociphilus aceris</i> | <i>Acer saccharinum</i> | Silver Maple | Aceraceae |
| 2 | <i>Paraprociphilus tessallatus</i> | <i>Alnus glutinosa</i> | European Alder | Betulaceae |
| 3 | <i>Paraprociphilus tessallatus</i> | <i>Alnus rugosa</i> | Speckled Alder | Betulaceae |
| 4 | Undescribed | <i>Alnus serrulata</i> | Hazel Alder | Betulaceae |
| 5 | Undescribed | <i>Echinocystis lobata</i> | Wild Cucumber | Cucurbitaceae |
| 6 | <i>Grylloprociphilus imbricator</i> | <i>Fagus grandiflora</i> | American Beech | Fagaceae |
| 7 | <i>Meliarhizophagus fraxinifolii</i> | <i>Fraxinus americana</i> | White Ash | Oleaceae |
| 8 | Undescribed | <i>Hamamelis virginiana</i> | Witchhazel | Hamamelidaceae |
| 9 | <i>Paraprociphilus tessallatus</i> | <i>Ilex verticillata</i> | Winterberry | Aquifoliaceae |
| 10 | <i>Neoprociphilus aceris</i> | <i>Smilax herbacea</i> | Carrion-flower | Smilacaceae |
| 11 | Undescribed | <i>Smilax hispida</i> | Bristly Greenbrier | Smilacaceae |
| 12 | Undescribed | <i>Malus pumila</i> | Common Apple | Rosaceae |
| 13 | Undescribed | <i>Ulmus</i> sp. | Elm | Ulmaceae |

(* From Scott 1986).



Figure 4.2. Final instar larva of F. tarquinius (Photograph by Darlyne Murawski).

Morphology

Myrmecophilous lycaenid larvae use an array of chemical, behavioral, and secretory cues to form and maintain associations with attendant ants that protect them from predators and parasites. These caterpillars may possess at least three organs assisting in this process: the dorsal nectary organ (DNO) found on the seventh abdominal segment, the tentacular organs (TOs), found on either side of the eighth abdominal segment and the pore cupolae organs (PCOs) found scattered dorsally along the length of the cuticle (Hinton 1951; Cottrell 1984). In the Miletinae, which is almost entirely myrmecoxenous, the DNO is lacking, and in most species, the TOs as well (Cottrell 1984). The PCOs however, do exist, though reduced in numbers relative to those in myrmecophilous counterparts (Cottrell 1984).

Sound

Larvae of Lycaenidae (including Riodininae) are capable of producing substrate-borne vibrations (DeVries 1990; 1991). With several exceptions [e.g. *Deudorix epijarbas* (De Baar 1984), *Caleta roxus* (Fiedler 1994)], most lycaenids that can produce vibrational

signals associate with ants as larvae (DeVries 1990; 1991, but see Downey 1966; Downey & Allyn 1978). Travassos & Pierce (2000) have demonstrated that larvae of the Australian lycaenid *Jalmenus evagoras* significantly increase call production in the presence of their attendant ants, and Millard, Wagner and Snedden (in preparation) have shown that calling by the North American lycaenid *Hemiargus isola* is important in attracting attendant ants.

The ability to produce vibrational signals appears to be widespread in the Lycaenidae (DeVries 1991; Fiedler et al. 1995; Heath 1998). In the first description of sound production in the Lycaenidae, Dodd (1916) mentioned *Miletus* among the genera he had heard produce calls as larvae. Without a species name, however, it is difficult to know whether the *Miletus* he was describing would be classified as a miletine today. Heath (personal communication) found that fourth instar larvae of *Thestor yildizae*, a South African miletine that lives in *Anoplolepis custodiens* ant nests, produces faint sounds in response to a disturbance.

MATERIALS AND METHODS

Life-history

Field surveys of *Alnus* spp. were undertaken across 14 site localities in New Brunswick, Maine, New Hampshire, and Massachusetts (Table 4.2) between June and September 1998, and between July and September 1999. Larvae at different instar stages found among the woolly aphids were collected, and at one site (the Arnold Arboretum), adults were also collected. The presence of ants attending the prey populations was noted, as were predators competing for the same prey base with the lycaenid larvae, such as syrphid flies (*Syrphus* spp.) (Figure 4.3) and neuropteran lacewings (*Chrysopa slossanae*). The larvae and associated predators and prey were collected and reared to adulthood under constant conditions (24°C) in a growth room. Wings were removed as voucher specimens and adult bodies were preserved in 100% ethanol and stored at -80°C.

Table 4.2 Location data for *F. tarquinius* in this study.

| No. | Date | Locality | Co-ordinates | Host aphid species | Attendant ant species (matched to tree (T)) | Host-plant |
|-----|---------|--------------------------------------|---------------------------|---|---|-------------------------------------|
| 1 | 4/9/98 | Phillipston, MA | 42°31'10"N, 72°08'00"W | <i>Paraprociophilus tessallatus</i> (Pemphigidae) | <i>Formica rubicunda</i> (Tree 1) (Formicinae) | <i>Alnus rugosa</i> (Betulaceae) |
| 2 | 7/9/98 | Petersham, MA | 42°29'15"N, 72°11'15"W | <i>Paraprociophilus tessallatus</i> | <i>Camponotus noveboracensis</i> , <i>Camponotus pennsylvanicus</i> , (Formicinae) | <i>Alnus rugosa</i> |
| 3 | 14/9/98 | Mt. Washington Regional Airport, NH | 44°22'03"N 71°32'40"W | <i>Paraprociophilus tessallatus</i> | No ants observed tending | <i>Alnus rugosa</i> |
| 4 | 15/9/98 | Berlin, NH | 44°28'07"N 71°11'08"W | <i>Paraprociophilus tessallatus</i> | <i>Formica fusca</i> (group) (Formicinae) | <i>Alnus rugosa</i> |
| 5 | 17/9/98 | Mt. Carleton Park, near Nictau, NB. | 47°14'00"N 67°09'00"W | <i>Paraprociophilus tessallatus</i> | <i>Myrmica incompleta</i> , <i>Formica fusca</i> (group) (Formicinae) | <i>Alnus rugosa</i> |
| 6 | 17/9/98 | Bathurst, NB | 47°25'00"N 65°55'00"W | <i>Paraprociophilus tessallatus</i> | <i>Formica fusca</i> (group) (Formicinae) | <i>Alnus rugosa</i> |
| 7 | 18/9/98 | St. Louis de Kent (Site 1), Kent, NB | 46°35'00"N 65°15'00"W | <i>Paraprociophilus tessallatus</i> | <i>Lasius pallitarsus</i> , <i>Formica integra</i> gp. (Formicinae) | <i>Alnus rugosa</i> |
| 8 | 18/9/98 | St. Louis de Kent (Site 2), Kent, NB | 46°35'00"N 65°15'00"W | <i>Paraprociophilus tessallatus</i> | <i>Camponotus noveboracensis</i> , <i>Formica fusca</i> (group) (Formicinae) | <i>Alnus rugosa</i> |
| 9 | 19/9/98 | Rt. 114, north of Fundy, NB | 45°37'00"N 65°02'00"W | <i>Paraprociophilus tessallatus</i> | <i>Formica fusca</i> (group) (Formicinae) | <i>Alnus rugosa</i> |
| 10 | 20/9/98 | Searsport, ME | 44°27'30"N 68°55'29"W | <i>Paraprociophilus tessallatus</i> | <i>Formica integra</i> , <i>Formica fusca</i> (group) (Formicinae) | <i>Alnus rugosa</i> |
| 11 | 3/7/99 | Arnold Arboretum, Boston, MA | 42°17'55"N 71°07'42"W | <i>Paraprociophilus tessallatus</i> | <i>Camponotus noveboracensis</i> , (Formicinae) <i>Myrmica rubra</i> (Myrmicinae) | <i>Alnus glutinosa</i> (Betulaceae) |
| 12 | 19/7/99 | Bar Harbor, ME | 44°23'45"N 68°11'38"W | <i>Paraprociophilus tessallatus</i> | No ants observed tending | <i>Alnus rugosa</i> |
| 13 | 28/7/99 | Petersham, MA | 42°29'15"N, 72°11'15"W | <i>Paraprociophilus tessallatus</i> | <i>Camponotus noveboracensis</i> , <i>Camponotus pennsylvanicus</i> , (Formicinae) | <i>Alnus rugosa</i> |
| 14 | 4/9/99 | Gorham (Site 1), NH | 44°30'89"N, 71°09'91"W | <i>Paraprociophilus tessallatus</i> | <i>Formica glacialis</i> , <i>Camponotus herculeanus</i> (Formicinae) | <i>Alnus rugosa</i> |
| 15 | 4/9/99 | Gorham (Site 2), NH | 44°31'85"N, 71°09'91"W | <i>Paraprociophilus tessallatus</i> | <i>Myrmica incompleta</i> (Myrmicinae) | <i>Alnus rugosa</i> |

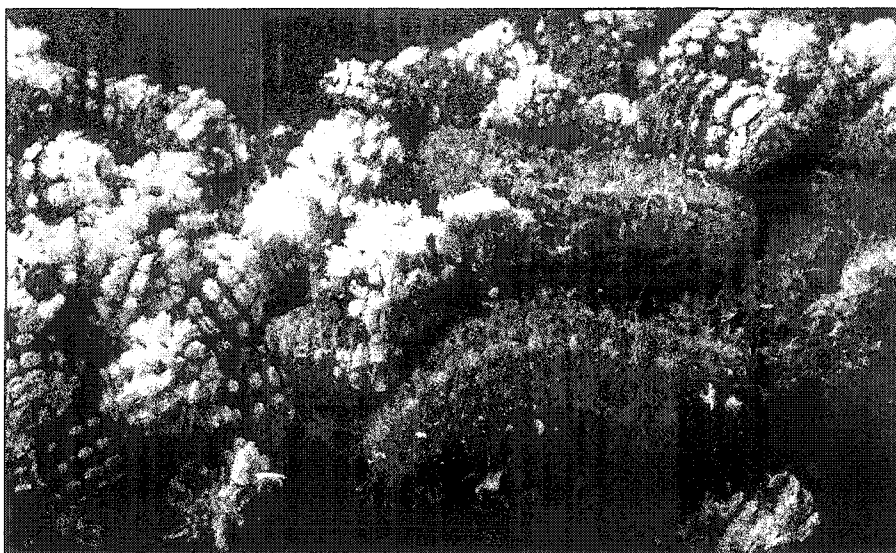


Figure 4.3. Grub of Syrphus (Diptera) and larva of Fenisea tarquinius (Photograph by Darlyne Murawski).

Morphology

The surface features of a single fourth instar *F. tarquinius* larva were studied under SEM (Figure 4.4). The sample was subjected to increasing levels of alcoholic dehydration (40%, 60%, 75%, 90%, 100%), prior to critical point drying with liquid carbon dioxide, employing a Tousimis Samdri PVT-3B. It was then mounted on a metal stub and sputter-coated with gold preparatory to viewing under SEM (JSM-6400).

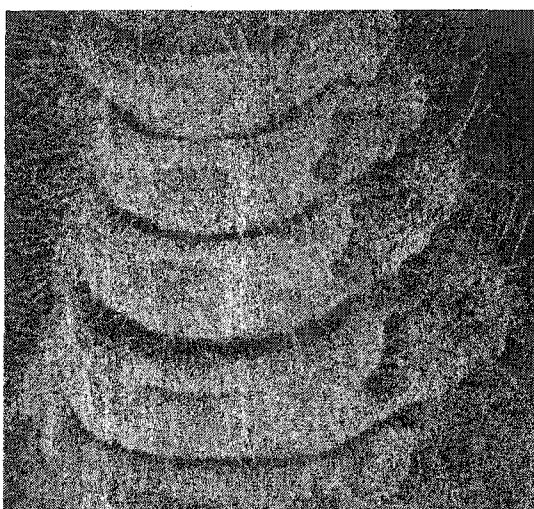


Figure 4.4. SEM photograph of upper dorsum of Fenisea tarquinius (18 X magnification).

The surface features of a single 4th instar *Taraka hamada* (Lycaenidae: Militinae: Spalgini) larva (Figure 4.5), the known closest relative to *F. tarquinius*, were also examined under the same conditions, except at higher resolution (32 X) for adequate discernment of features.

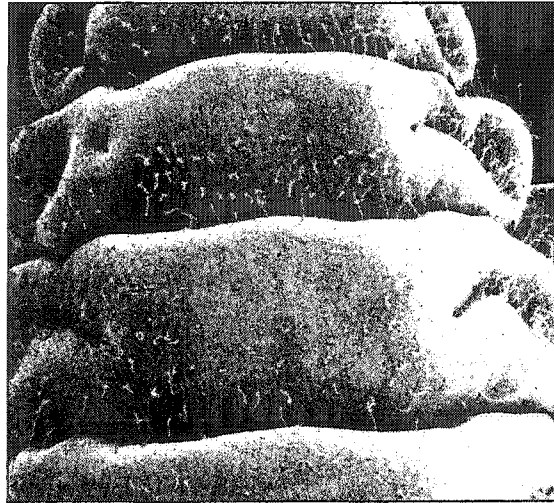


Figure 4.5. SEM photograph of upper dorsum of Taraka hamada (32 X magnification).

Sound

Several late instar larvae of *F. tarquinius* were collected in June 1999, in the Arnold Arboretum in Jamaica Plain, Massachusetts. In the laboratory, each larva was placed on a recording stage that consisted of a paper circle with a 12 cm diameter tightly taped over the opening of an 8 cm tall, 12 cm diameter plastic container so that the stage was taut. A Pfanstiehl P-136 Sonotone 2T phonograph cartridge taped to the recording stage acted as a microphone. Calls were recorded on a Nagra IV-SJ Tape Recorder with maximum gain. We induced larvae of *F. tarquinius* to call by stimulating them with a fine-haired paintbrush. We did not monitor sound production in a more natural context such as when *F. tarquinius* feeds on ant-tended woolly aphids.

Calls were examined with Canary 1.2b 1994, a sound analysis program produced by the Cornell Laboratory of Ornithology (Figure 4.6). The beginning and end of a call were defined with respect to the background noise level. At least ten call samples were taken

for each larva examined, and 33 calls from three larvae were analyzed in total. For each call, three properties were measured: mean dominant frequency, bandwidth, and pulse length. The dominant frequency was calculated as the average of the upper and lower frequency bounds of a call. The bandwidth of a call consisted of the difference between these upper and lower bounds. The pulse length was measured as the duration of a call. Summary statistics are reported as mean \pm one standard error, and counts given refer to the number of larvae sampled.

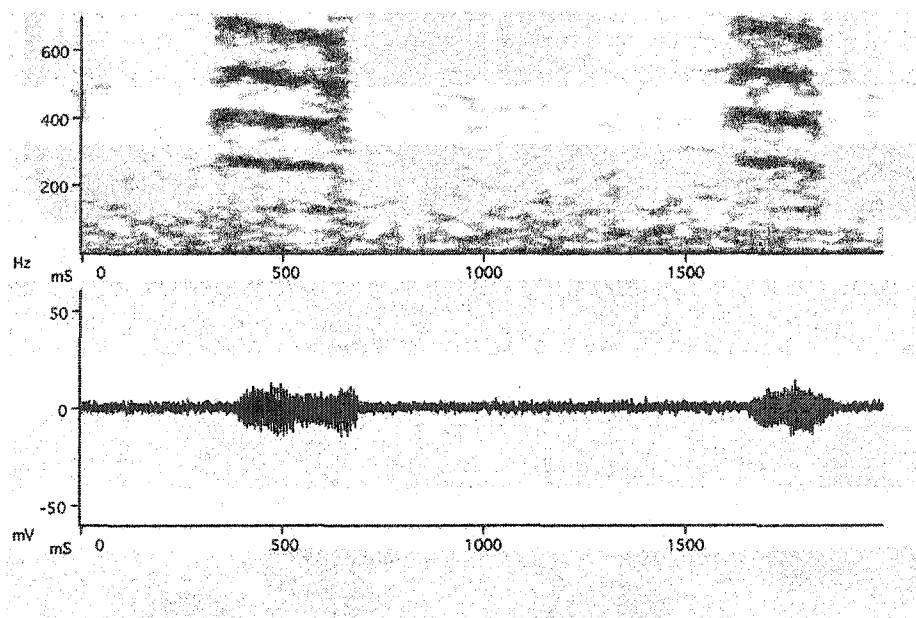


Figure 4.6. Larval calls. Pulse width vs. a) frequency b) bandwidth.

RESULTS

Life-history

Aphids ranged from a few individuals to dense clusters. At 14 site localities (Table 4.2), the host aphids (*Paraprociphilus tessallatus*) were found on *Alnus rugosa*, (Figure 4.2) and at one site, they occurred on *Alnus glutinosa*. The larvae were typically found burrowing under the aphids, out of reach of the attendant ant-guard. Adults were

encountered only four times; on one occasion, a female was seen to extrude what appeared to be a scent gland on the surface of a leaf (Figure 4.7).

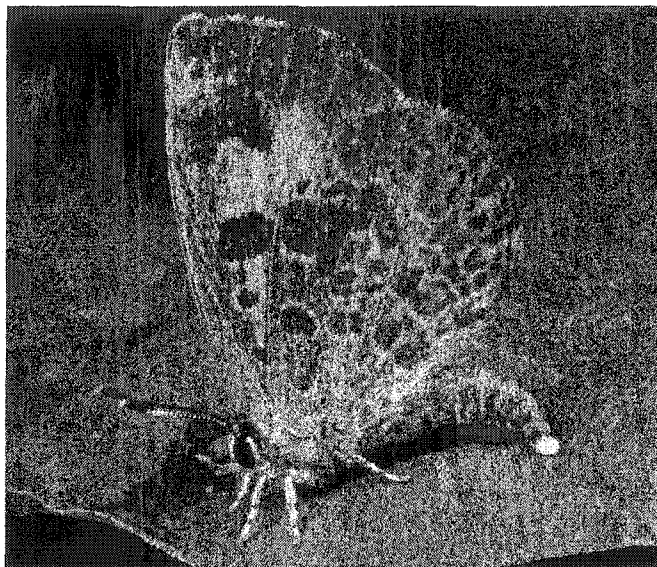


Figure 4.7. Adult of *F. tarquinius* extruding scent gland on the surface of a leaf.

Morphology

SEM examination of the surface of the *F. tarquinius* larva at 18X magnification revealed a hirsute dorsum replete with setae, and a few scattered PCOs along its length. There was no evidence of a DNO or TOs (Figure 4.4). Likewise, *T. hamada* possessed scattered PCO's, but no visible DNO's or TOs (Figure 4.5).

Sound

Larvae of *F. tarquinius* produced a call that, depending on the distance from the phonograph cartridge, resembled a mournful sigh, a falling *glissando* of six half-steps from F to middle C, or, at proximity, when amplified, the bleating of a sheep. Larvae called when disturbed with a paintbrush. When calling, a caterpillar lifted its anterior portions, including its head and thorax, off the substrate. The larval call (N=3) of *F. tarquinius* had a mean dominant frequency of 302.1 ± 29.1 Hz, a pulse length of $477.0 \pm$

143.2 ms, and a bandwidth of 79.3 ± 8.4 Hz (Figure 4.7). In a typical larval call, there was a drop in frequency over the length of the call.

DISCUSSION

Life-history

Banno (1997) showed that a close relative of *F. tarquinius* in the Palaearctic, *Taraka hamada*, was responsible for decimating whole colonies of its host aphid, *Ceratovacuna japonica*. In our observations, the larvae of *F. tarquinius* appeared to be remarkably similar to *T. hamada*. In both cases, competing predators are often present; syrphid flies in numbers, and more infrequently, lacewings. The ant species tending the prey aphids of *F. tarquinius* in 13 of 15 cases belonged to the Formicinae; this may be a function of the disproportionate abundance of formicine species in the north temperate region (Stefan Cover personal communication). The only exceptions to this pattern involved two observations of tending of aphids by *Myrmica rubra* (Myrmicinae), which is an exotic species introduced in the early part of the century from Europe (Stefan Cover personal communication).

The biocontrol potential of *F. tarquinius* was suggested by Brower (1947) who reported that in Indian Town, Maine, the destructive balsam woolly aphid, *Adelges piceae*, was preyed upon by a dozen larvae of *F. tarquinius*. This was, however, a single record for the phenomenon, and little evidence of economic importance has since emerged. Another close relative in the Spalgini, *Spalgis*, however, does play a significant biocontrol role in keeping populations of several species of the Pseudococcidae in check, particularly those belonging to the genera *Phenacoccus*, *Planococcoides* and *Pseudococcus* (Ackery 1990).

The function of the extrusion by the adult female of *F. tarquinius* (Figure 4.7) on the surface of a leaf remains unclear. One possibility is that she may be extruding a pheromone to attract mates, but in the absence of more conclusive evidence, this must remain speculative. She was observed to expose the putative organ and retract it several times during ten minutes of observation. This indicated that it was, indeed, an extrusible gland and not a meconial defecation.

Morphology

It is unsurprising that the SEM examination of *F. tarquinius* revealed few surface structures, given such a trend overall for the Miletinae (Cottrell 1984). Compared to *T. hamada*, which was also examined under SEM (Figure 4.5) the dorsum of *F. tarquinius* larvae possesses many dorsal setae, and it may be that these play a defensive role against predatory attack. Hinton (1951) suggests that the waxy secretions of the aphid prey become entangled among the dorsal setae of *F. tarquinius*, and this, along with the web constructed by the larva, protects it against ants.

Sound

This is the first experimental demonstration of a stridulatory signal in a miletine, although Heath (personal communication) observed that fourth instar larvae of *Thestor yildizae*, a South African miletine that lives in *Anoplolepis custodiens* ant nests, produce faint sounds in response to a disturbance. Like other lycaenids, *F. tarquinius* larvae produce substrate-borne vibrations. However, their calls are distinctive in two ways. The pulse train is almost five times longer than the grunt of *Jalmenus evagoras*, previously the longest reported call of a lycaenid (Travassos & Pierce 2000), although the length of each pulse is roughly comparable to that of *J. evagoras*. In addition, the calls possess the narrowest bandwidth reported for lycaenid caterpillars. Unlike most lycaenid calls, which typically are short, broad bandwidth pulses (DeVries 1991), *F. tarquinius* larvae produce calls that have a high level of structure, suggesting that they may have a well-defined function. Qualitatively, these larval calls resemble the sounds produced by certain ant-tended membracids (Cocroft 1996; Mark Travassos personal observation). It is possible that *F. tarquinius* larvae may mimic the acoustical signals produced by the woolly aphids upon which they feed. It is not known, however, whether such aphids, like other ant-tended homopterans, produce substrate-borne vibrations. Non-ant-tended woolly aphids were silent when monitored.

Although most lycaenids that produce vibrational signals (customarily as later instars) associate with ants as larvae, Downey and Allyn (1978) did not find a strict correlation

between ant association and sound production in lycaenid pupae. Downey (1966) reported hearing sound produced by pupae of *F. tarquinius*, which are far less tended than larvae in the species. The demonstration of sound production in *F. tarquinius* larvae was conducted in the laboratory without the presence of attendant ants. Our attempts to induce sound production in *F. tarquinius* pupae were unsuccessful. Further observations monitoring call production in a natural setting may shed light on the role of sound in the larval and pupal stages of *F. tarquinius*, particularly its potential role in mimicry and ant association.

SECTION 5

CONSERVATION IN THE LYCAENIDAE WITH SPECIAL REFERENCE TO APHYTOPHAGOUS FORMS

Previous sections have focused on evolutionary and ecological aspects of the Miletinae (Lepidoptera: Lycaenidae) and as a closing chapter, it may be prudent to pay some attention to long-term conservation problems and strategies for the subfamily. Conservation paradigms are largely rooted in issues of public policy, and resultant decisions are increasingly being formulated in terms of habitat conservation with the species under threat becoming 'umbrella species' for other elements of the biota located in those habitats. No miletine species, however, has as yet been treated in this light, and it is hence instructive to examine the larger issues of lycaenid conservation strategies and even butterfly conservation as a whole, abstracting from issues of general conservation concern to the specifics of aphytophagous lycaenids, miletine or otherwise. In so doing, concepts integral to concepts of conservation practice shall be discussed.

The organizational scheme for this chapter is as follows:

- a) A brief overview of lycaenid biology
- b) The 2002 IUCN Red-data listing of lycaenids of special concern
- c) Suggested causes of decline for butterflies
- d) Conservation measures for Lycaenidae – the importance of surrogate species
- e) Three case studies

A brief overview of lycaenid biology

The Lycaenidae (blues, coppers, hairstreaks, metalmarks and harvesters) are the most diverse family of Papilionoidea, comprising between 30 and 40% of all butterfly species. They tend to be small, with the world's tiniest butterflies known possibly being *Brephidium exilis* (Boisduval) and *Micropsyche ariana* with wingspans of only 6 and 7mm respectively (New 1993, 1997a). The largest known lycaenid is the miletine species, *Liphyra brassolis*, with a wingspan of close to 9 cm (New 1993). The family

occurs in all major biogeographical regions in both temperate and tropical zones. Many species have very precise environmental requirements, but the family occurs in major biomes and vegetation associations from climax forests to scrublands, grasslands, wetlands and semi-arid desert communities. Some lycaenids have considerable potential for use as indicator species as their incidence and abundance reflects small degrees of habitat change (New 1993).

Many lycaenid larvae feed on flowerbuds, flowers and fruits (Downey 1962), and therefore they may exert stronger selective pressures on their foodplants than many foliage feeders (Breedlove & Ehrlich 1968). These pressures in turn may have largely driven the departure of many lycaenids from customary lepidopteran dependence on angiosperm and gymnosperm plants, so as to feed on other groups such as fungi or animals, a condition collectively termed aphytophagy. The extent of aphytophagy, including predatory and mutualistic relationships with ants and several homopteran species, can be either facultative or mutualistic (Cottrell 1984; Pierce 1995), such that lycaenids, as a group, participate in a wider range of ecological interactions than any other group in the Lepidoptera. Very few lycaenids are widely distributed, although exceptions include *Lampides boeticus* that extends from Europe to Australia and Hawaii, *Celestrina argiolus*, which is found throughout most of the Palaearctic, Oriental and Nearctic regions (New 1995). The reason for this restricted distribution appears to be that most lycaenids are not particularly vagile, and many cannot cross even small spaces between habitat patches. Such restriction renders the Lycaenidae of immediate conservation concern.

The specialization to ant-attendance among several lycaenids is another feature that renders such species potentially vulnerable; this will be discussed at greater length under the section: 'causes for decline.' The possible advantages of enemy-free space that ants afford have been discussed by Atsatt (1981); Pierce and Mead (1981); Henning (1983); Cottrell (1984); Pierce (1984); Pierce and Eastal (1986); and De Vries (1991a); in return for which, the ants obtain additional food from caterpillars (Pierce & Mead 1981, Fiedler & Maschwitz 1988; Baylis & Pierce 1992). Mutualism with ants may have been an early development in lycaenid evolution (Eliot 1973). Pierce (1984) posited that lycaenid

diversity may reflect speciation events that could be influenced by larval/ant associations in two ways:

- a) Female lycaenids may adopt ants as oviposition cues so that the presence of ants on a novel foodplant may induce a rapid host switch. Essentially, novel foodplant choices may be made by female lycaenids to an unusually high degree because they select for ants as well as chemically and physically suitable foodplants.
- b) Small semi-isolated populations with little regular genetic interchange between may result in, rather than be caused by, the general non-vagility of many lycaenids. Classic metapopulational studies on butterflies have largely involved species of the family Nymphalidae that are decidedly non-vagile [e.g. the studies of Murphy et al. (1990) on the Bay Checkerspot Butterfly, *Euphydryas editha bayensis*, and those of Britten et al. (1994) on the Uncompahgre Fritillary Butterfly, *Boloria acrocnema*]. The observed patterns, however, can be extended to several lycaenids as well (e.g. the Fender's blue butterfly, *Icaricia icarioides fenderi*, where Schultz in 1996 showed that the pattern of dispersal was only 0.75 km in its primary lupine habitat patches and no more than 2 km between non-lupine patches).

Larval feeding is further complicated when aphytophagy is involved (Table 5.1), because the prey item is as much a limiting factor as its host-plant. This may well be a pointer as to why there are so few aphytophagous butterfly species.

Table 5.1 Aphytophagous lycaenid larvae (revised from Eliot 1973; Cottrell 1984; New 1993).

| <i>Subfamily</i> | <i>Number of Tribes</i> | <i>Number of Genera</i> | <i>Genera with all or some aphytophagous species</i> |
|------------------|-------------------------|-------------------------|---|
| Poritiinae | 3 | 53 | None |
| Miletinae | 4 | 13 | <i>Liphyra</i> , <i>Euliphyra</i> , <i>Aslauga</i> , <i>Miletus</i> , <i>Allotinus</i> , <i>Megalopalpus</i> , <i>Taraka</i> , <i>Spalgis</i> , <i>Feniseca</i> , <i>Lachnocnema</i> , <i>Thestor</i> |
| Curetinae | 1 | 1 | None |

Table 5.1 (continued)

| <i>Subfamily</i> | <i>Number of Tribes</i> | <i>Number of Genera</i> | <i>Genera with all or some aphytophagous species</i> |
|------------------|-------------------------|-------------------------|---|
| Lycaeninae | 24 | 408 | <i>Acrodipsas, Shirozua, Zesius, Spindasis, Oxychaeta, Trimenia, Argyrocupha, Triclina, Niphanda, Maculinea, Lepidochrysops</i> |

Relatively few species of Lycaenidae seem to be marked by a polyphagous habit and many have very restricted ranges of foodplants (New 1993). For instance, in any one area, only a single plant genus is used for oviposition (Cottrell 1984). Rarely, different generations of the same species may utilize different foodplants: the first (spring) generation of *Celastrina argiolus* in Europe eats holly, while the second (summer) generation eats ivy. Nitrogen-rich plant feeding is also a characteristic of many lycaenid taxa (Pierce 1985).

The 2002 IUCN Red Data List of lycaenids of special concern

In the 2002 IUCN Red Data Book of Threatened Animals, there are 100 lycaenid species listed (Table 5.2). Many endangered lycaenids, including celebrated examples, merit subspecific rather than specific rank, and hence do not find inclusion in the table below. In addition, several countries have their own national lists of threatened lycaenid taxa, the most enveloping, and consequently, least discriminating being India, with 162 listed species (only 4 of which are putatively aphytophagous). These figures may appear to outstrip the IUCN numbers, but it is important to note that species that are at risk at a national level may be more secure globally, on account of viable population sizes elsewhere.

Table 5.2 Lycaenid species included on the 2002 IUCN Red List of Threatened Animals

(Note: species with asterisks indicate actual or putative aphytophagy).

| <i>Taxa</i> | <i>Status</i> | <i>Country</i> |
|-----------------------------------|-----------------------|---|
| <i>Acrodipsas illidgei</i> * | Endangered | Australia |
| <i>Agriades zullichi</i> | Endangered | Spain |
| <i>Alaena margaritacea</i> | Vulnerable | South Africa |
| <i>Aloides caledoni</i> | Vulnerable | South Africa |
| <i>Aloides carolynnae</i> | Vulnerable | South Africa |
| <i>Aloides dentatis</i> | Vulnerable | South Africa |
| <i>Aloides egerides</i> | Vulnerable | South Africa |
| <i>Aloides kaplani</i> | Vulnerable | South Africa |
| <i>Aloides lutescens</i> | Vulnerable | South Africa |
| <i>Aloides merces</i> | Vulnerable | South Africa |
| <i>Aloides nollothi</i> | Vulnerable | South Africa |
| <i>Aloides nubilus</i> | Vulnerable | South Africa |
| <i>Aloides pringlei</i> | Vulnerable | South Africa |
| <i>Aloides rossouwi</i> | Vulnerable | South Africa |
| <i>Arawacus aethesa</i> | Endangered | Brazil |
| <i>Aslauga australis</i> * | Vulnerable | South Africa |
| <i>Capys penningtoni</i> | Endangered | South Africa |
| <i>Chrysoritis cotrelli</i> | Critically Endangered | South Africa |
| <i>Chrysoritis oreas</i> | Lower Risk | South Africa |
| <i>Cyanophrys berthia</i> | Vulnerable | Brazil |
| <i>Deloneura immaculata</i> | Extinct | South Africa |
| <i>Erikssonia acraeina</i> | Vulnerable | Democratic Republic of the Congo, South Africa, Zambia |
| <i>Glaucopsyche xerces</i> | Extinct | U.S.A. |
| <i>Iolaus aphnaoides</i> | Lower Risk | South Africa |
| <i>Iolaus lulua</i> | Vulnerable | South Africa |
| <i>Joiceya praeclarus</i> | Endangered | Brazil |
| <i>Lepidochrysops bacchus</i> * | Lower Risk | South Africa |
| <i>Lepidochrysops badhami</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops balli</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops hypopolia</i> * | Extinct | South Africa |
| <i>Lepidochrysops jefferyi</i> * | Vulnerable | South Africa |

Table 5.2. (continued)

| <i>Taxa</i> | <i>Status</i> | <i>Country</i> |
|---------------------------------------|-----------------------|--------------------------|
| <i>Lepidochrysops littoralis</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops loewensteini</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops lotana</i> * | Critically Endangered | South Africa |
| <i>Lepidochrysops oosthuizen</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops outeniqua</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops penningtoni</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops pephredo</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops poseidon</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops pringlei</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops quickelbergei</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops swanepoeli</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops titei</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops victori</i> * | Vulnerable | South Africa |
| <i>Lepidochrysops wykehami</i> * | Vulnerable | South Africa |
| <i>Lycaena dispar</i> | Lower Risk | Europe, Central Asia |
| <i>Lycaena hermes</i> | Vulnerable | Mexico, U.S.A. |
| <i>Lycaena ottomanus</i> | Vulnerable | South and Central Europe |
| <i>Maculineaalcon</i> * | Lower Risk | Europe, Central Asia |
| <i>Maculinea arion</i> * | Lower Risk | Europe, Central Asia |
| <i>Maculinea arionides</i> * | Lower Risk | China, Japan, Russia |
| <i>Maculinea nausithous</i> * | Lower Risk | Europe, Central Asia |
| <i>Maculinea rebeli</i> * | Vulnerable | South and Central Europe |
| <i>Maculinea teleius</i> * | Lower Risk | Europe, Northern Asia |
| <i>Nirodia belphegor</i> | Endangered | Brazil |
| <i>Orachrysops ariadne</i> * | Vulnerable | South Africa |
| <i>Orachrysops niobe</i> * | Endangered | South Africa |
| <i>Oxychaeta dicksoni</i> | Endangered | South Africa |
| <i>Paralucia spinifera</i> | Endangered | Australia |
| <i>Phasis pringlei</i> | Vulnerable | South Africa |
| <i>Plebeius hesperica</i> | Vulnerable | Spain |
| <i>Plebeius trappi</i> | Vulnerable | Italy, Switzerland |
| <i>Poecilmitis adonis</i> | Vulnerable | South Africa |
| <i>Poecilmitis aureus</i> | Lower Risk | South Africa |
| <i>Poecilmitis azarius</i> | Vulnerable | South Africa |
| <i>Poecilmitis balli</i> | Vulnerable | South Africa |

Table 5.2. (continued)

| <i>Taxa</i> | <i>Status</i> | <i>Country</i> |
|--------------------------------|-----------------------|----------------|
| <i>Poecilmitis daphne</i> | Vulnerable | South Africa |
| <i>Poecilmitis endymion</i> | Vulnerable | South Africa |
| <i>Poecilmitis henningi</i> | Vulnerable | South Africa |
| <i>Poecilmitis hyperion</i> | Vulnerable | South Africa |
| <i>Poecilmitis irene</i> | Vulnerable | South Africa |
| <i>Poecilmitis kaplani</i> | Vulnerable | South Africa |
| <i>Poecilmitis lyncurium</i> | Vulnerable | South Africa |
| <i>Poecilmitis lyndseyae</i> | Vulnerable | South Africa |
| <i>Poecilmitis orientalis</i> | Vulnerable | South Africa |
| <i>Poecilmitis pan</i> | Data Deficient | South Africa |
| <i>Poecilmitis penningtoni</i> | Vulnerable | South Africa |
| <i>Poecilmitis pyramus</i> | Vulnerable | South Africa |
| <i>Poecilmitis rileyi</i> | Endangered | South Africa |
| <i>Poecilmitis stepheni</i> | Vulnerable | South Africa |
| <i>Poecilmitis swanepoeli</i> | Endangered | South Africa |
| <i>Poecilmitis trimeni</i> | Vulnerable | South Africa |
| <i>Poecilmitis wykehami</i> | Vulnerable | South Africa |
| <i>Polyommatus dama</i> | Endangered | Turkey |
| <i>Polyommatus galloi</i> | Endangered | Italy |
| <i>Polyommatus golgus</i> | Endangered | Spain |
| <i>Polyommatus humedasa</i> | Critically Endangered | Italy |
| <i>Strymon avalona</i> | Vulnerable | U.S.A. |
| <i>Thestor brachycerus</i> * | Lower Risk | South Africa |
| <i>Thestor compassbergae</i> * | Vulnerable | South Africa |
| <i>Thestor dryburghi</i> * | Vulnerable | South Africa |
| <i>Thestor kaplani</i> * | Vulnerable | South Africa |
| <i>Thestor pringlei</i> * | Vulnerable | South Africa |
| <i>Thestor rossouwvi</i> * | Vulnerable | South Africa |
| <i>Thestor stepheni</i> * | Vulnerable | South Africa |
| <i>Thestor strutti</i> * | Vulnerable | South Africa |
| <i>Thestor swanepoeli</i> * | Vulnerable | South Africa |
| <i>Thestor tempe</i> * | Vulnerable | South Africa |
| <i>Thestor yildizae</i> * | Vulnerable | South Africa |
| <i>Trimenia wallegrenii</i> * | Endangered | South Africa |

(Explanation of IUCN terminology employed in Table 5.2:

Extinct: A taxon is Extinct when there is no reasonable doubt that the last individual has died.

Critically Endangered: A taxon is Critically Endangered when it is facing an extremely high risk of extinction in the wild in the immediate future.

Endangered: A taxon is Endangered when it is not Critically Endangered but is facing a very high risk of extinction in the wild in the near future,

Vulnerable: A taxon is Vulnerable when it is not Critically Endangered or Endangered but is facing a high risk of extinction in the wild in the medium-term future,

Lower Risk: A taxon is Lower Risk when it has been evaluated, does not satisfy the criteria for any of the categories Critically Endangered, Endangered or Vulnerable. Included are taxa which are the focus of a continuing taxon-specific or habitat-specific conservation programme targeted towards the taxon in question, the cessation of which would result in the taxon qualifying for one of the threatened categories above within a period of five years.

Data Deficient: A taxon is Data Deficient when there is inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status.)

Table 5.2 reveals a high number, 77/100, of listed species from South Africa. The current 77% contrasts to 48% in 1990, when 37 of 77 species, some since removed from consideration, were South African. This figure is likely to result from a combination of three factors a) a function of pronounced endemism b) sampling effort c) and a marked propensity on the part of many South African lepidopterists to name species indiscriminately. The last can skew figures tremendously, and hence the figures must be treated with some caution. It is pertinent to note that from this listing, Neotropical species are conspicuously and perhaps artificially few, which may result from inadequate sampling from that biogeographic region. Equally puzzling is the total absence of Oriental species; in the 1990 list, there were two Indian species listed (again, an abnormally low number). Given the diversity of lycaenid fauna in South and South-East Asia, and the tremendous pressure of habitat destruction ensuing from the pressures of burgeoning human populations, the paucity of Oriental lycaenids in the current Red Data List is likely anomalous. Similar to the pattern for the Neotropics, it probably reflects poor sampling. South Africa itself is, with one exception, the sole contributor to the Afrotropical representation on the list, indicating that sampling efforts elsewhere in that biogeographic region are few. As in South Africa, sampling effort has typically been

intense in Northern Europe, the United States and Australia. The number of endemic species in the Holarctic and Australian regions is comparatively lower than in the Afrotropical and Oriental region, so a relatively low percentage of species in the former areas cannot be treated on par with those of the latter.

Of the 100 species listed, 40 are actually or presumably aphytophagous or phytopredaceous (40 %) (Table 5.2). The species of *Maculinea* (6/6 i.e. 100% inclusion) have been the focus of far more intense study than the over 120 species of the putatively phytopredaceous South African genus *Lepidochryops* (19 species of which are deemed endangered by the IUCN i.e. 16%; please note, however, that *Orachrysops*, which is putatively phytopredacious is closely related to *Lepidochryops* and was until recently, considered such), and the 29 species of the South African genus *Thestor* (11 of which are deemed endangered, i.e. 38%, although communications from my collaborators suggest that extreme restriction of range for several more might render them also of particular risk).

Suggested causes for decline

Collins and Morris (1985) enumerate four major processes that have led directly to a decline in swallowtail butterflies (Family Papilionidae). These processes are as valid for any group of butterflies and are given below in relation to lycaenids:

- a) Collecting and trade
- b) Pollution
- c) Exotic introductions
- d) Habitat destruction and alteration

a) Collecting and trade: The effects of collecting are controversial and difficult to assess. Since lycaenids occur in small, closed populations, they may be much more vulnerable to localized collector pressure. It is suggested that the Large Copper (*Lycaena dispar*) in Great Britain went extinct in part due to increased collector pressure on populations that had been already rendered vulnerable by habitat destruction (New 1993). In most cases, collecting pressure is probably the subsidiary rather than the prime cause.

Importantly, specialist collection to help monitoring of restricted colonies for conservation can be of direct damage to populations; Murphy (1989) showed that conventional mark-recapture studies used to estimate population sizes of taxa at risk could cause inadvertent damage through handling or induced changes in individual behavior. Total collecting bans are difficult to enforce. Kudrna (1989) indicated that in Germany, a ban on the collection of four butterfly species passed in 1936 has not prevented the decline of those species in 55 years. Even private collectors who obey voluntary restrictive quotas can cause harm if in large numbers, since they are unable to recognize when they are depleting butterfly stocks below the threshold of recovery (Collins & Morris 1985). Munguira et al. (1993), however, point out in their assessment of European lycaenids that in large populations, the number of butterflies a collector can take is negligible, not reaching 10% of the total daily population estimates, while small populations are typically of little interest to commercial collectors. Fortunately, lycaenids do not suffer from collection pressure the way more showy papilionids or nymphalids might, either for commercial pinned display or as exhibits in butterfly houses.

b) Pollution and pesticides: The effects of chemical pollution on lycaenids are difficult to assess, but it is conceivable that the decline of a number of species is in part due to such adverse elements as acid rain. Likewise, the effects of pesticides, though not quantified, have also been implicated. Balletto et al. (1982) found considerable differences between butterfly communities in heavily sprayed and traditionally managed rice fields. While on average five butterfly species were observed on each of the relatively natural habitats which supported *Lycaena dispar*, only two species could be found in the more heavily sprayed fields and *L. dispar* was entirely absent. Similarly, Dover et al. (1990) in a survey extending over four years, showed that conservation headlands (i.e. areas demarcated for conservation in lands put to multiple use), hosted more butterfly species than those found in normally sprayed fields. Dover (1991) posited that conservation headlands may help to protect hedges and field boundaries against spray drift; however, the overall conservation value of headlands could depend on allowing the growth of a wide range of larval food plants, rather than using herbicides. Brower (1995) predicted that the unregulated use of herbicides could well lead to the decline of the Monarch butterfly (*Danaus plexippus*) since the goal of killing all

competing plants over tens of millions of hectares of croplands would reduce the 'weedy' food plants as a direct consequence. Davis (1965) noted that general broad-spectrum pesticide applications threatened species of conservation concern the most, because destruction of even a few individuals could decrease the breeding population disproportionately.

c) Exotic introductions: The effects of exotic introductions upon local lycaenid communities have tended to be indirect. In the 1950's, the introduction of myxomatosis in Britain led to a reduction of the intensity of rabbit grazing on chalk grasslands, resulting in reduced numbers of several butterfly species, including *Lysandra bellargus* and *Maculinea arion* (New 1993) (the latter case is discussed further under habitat alteration and fragmentation). Another adverse effect of exotic introductions is the potential for hybridization and loss of species distinctness (New 1991; Scoccimarro 1995). By contrast, Brown (1990) showed that the widespread species *Leptotes marina* (Reakirt) had adapted well to urbanization in North America with its range expansion mainly due to a switch to a South African larval foodplant (*Plumbago auriculata*), which is used in freeway landscaping and as an ornamental.

Other effects of generalized predators are also important, if not quantified extensively. For instance, the Argentine Ant, *Linepithima* (earlier *Iridomyrmex*) *humile* has overrun much of South Africa and Henning and Henning (1992) consider it probably the most damaging alien introduction, since by out-competing native ant species, it may affect myrmecophilous lycaenids. Samways (1993) also points out that *L. humile* does not disperse and bury seeds like the native ant species, which will eventually result to the alteration of the habitat through loss of the native Cape Proteaceae species through slow attrition of seed reserves.

d) Habitat alteration and fragmentation: By far the greatest threat to all lycaenid species with low vagility and limited distribution, this factor has been widely implicated in the case studies of the major declines of some celebrated lycaenids. Lycaenid taxa are particularly susceptible to certain types of habitat alteration including changes in forestry practices in tropical and temperate regions; the conversion of shrub-land to agricultural; wetland drainage; heathland succession; grassland management, grazing, and expanding urban, industrial and recreational land use (New 1993). For instance, in Europe,

calcareous grassland is a particularly important lycaenid habitat that has undergone large-scale changes. The extinction of *Cyaniris semiargus* (Rottemburg) in Britain in 1877 is often attributed to changes in grassland management (Heath 1981). Other celebrated extinctions in Europe have included *Lycaena dispar* and the aphytophagous *Maculinea arion* in the United Kingdom (Duffey 1968; Thomas 1980), and *Lycaena hippothoe*, and the aphytophagous species *Maculinea arion*, *Maculinea nausithous* and *Maculinea teleius* in the Netherlands (Heath 1981).

Habitat alterations can be quite subtle: a slight change of vegetational growth for *Maculinea arion* sites in Britain will suffice to render the habitat unsuitable (Thomas 1989). Ironically, this change was produced by grazing relaxation. The rapid decline of extensive grazing over almost all of Europe due to intensified agricultural practices and abandonment of former grazing areas is particularly devastating for lycaenids that have co-existed with humans for millennia and have probably benefited from the patchy structure of agrobiosystems in which grasslands, hedges and woodlands occur together (Munguira et al. 1993). Leopold's (1949) summary moral maxim bears mention in this context, "A thing is right when it tends to preserve the integrity, stability and beauty of the biotic community. It is wrong when it tends otherwise."

Als (2001) indicates that approximately one third of all lycaenid species that are listed on the IUCN Red Data List of Threatened Species are entomophagous (or more broadly, aphytophagous) (Table 5.2), and he believes that with the exception of *Lepidochrysops*, the entomophagous lifestyle is a short-lived experiment. Several causes are advanced for this including phylogenetic constraint (Pierce et al. 2002), population fragmentation, caused by the requirement of an overlapping mosaic of ant and plant distribution, thereby increasing the risk of local extinction (Pierce et al. 2002), and the complexity of the life cycles involved. Habitat availability and quality are thereby key features (New et al. 1995; Hanski & Simberloff 1997, Clarke et al. 1997), with habitat quality affecting local population dynamics within a patch, and habitat availability affecting persistence at a regional level (Als 2001).

To counter the effects of habitat fragmentation, dispersal is a prerequisite for the persistence of isolated populations. The input of individuals from neighboring areas can bolster populations whose numbers are dwindling, thereby preventing their extinction i.e.

the 'rescue effect' (Brown & Kodric-Brown 1977). Dispersal can also provide an influx of genetically variant individuals into a population, thereby increasing genetic diversity, and hence greater fitness and population viability (Vrijenhoek 1985). Taxa with limited dispersal ability are hence far more susceptible to local extinction events than taxa with well-developed dispersal abilities.

In their comprehensive study of the effect of habitat fragmentation on the threatened nymphalid *Euphydryas editha editha* (the Bay checkerspot butterfly) of California, while conducting population viability assessments on the species, Murphy et al. (1990) show that their assessments follow an environmental metapopulation approach. Variations in thermal conditions and precipitation through time (macroclimatic factors) and across local topography (topoclimate) drive the population dynamics of the insect. On account of the great sensitivity of the larvae to these factors, both habitat patch quality and distance from a reservoir population affect the likelihood of patch occupancy. An understanding of metapopulational dynamics is necessary to explain the regional distribution of the butterfly at any given time.

Shaffer (1981; 1985) identifies four categories of phenomena affecting the persistence of all populations:

a) Genetic factors that negatively affect the ability of individuals to survive and reproduce, and the ability of populations to adapt to changing environments.

b) Demographic factors that affect population size and persistence, including such factors as sex ratio, reproductive output and age at first reproduction. To this may also be added specialization.

c) Environmental factors including changes in climate and other habitat characteristics with other largely stochastic processes that affect the availability of key resources for a particular population, as do natural catastrophes such as fire, flood and intense and/or sustained drought.

d) Local population interactions or metapopulation dynamics, which affect levels of gene flow between populations, patterns of habitat patch occupancy and recolonization rates following local extinction events.

Naturally, these factors can work in concert to produce amplified effects. Gilpin and Soulé (1986) have described the consequences of the phenomena that affect population

persistence as 'extinction vortices.' Feedback loops among genetic, environmental, demographic and environmental phenomena determine the vulnerability of populations to extinction. This is certainly true of the large blue, *Maculinea arion* in Britain as suggested earlier. *Maculinea arion* already suffered from low population sizes, and hence demographic stochasticity played a key role (Thomas 1984), in synergy with profound environmental factors such as grazing relaxation. During early instars, the *M. arion* larvae feed on wild thyme (*Thymus drucei praecox*) and at the fourth instar, are carried by *Myrmica* ants into their nests, where the lycaenids feed on ant brood. The level of grazing in the grassland habitats was progressively reduced from 1950, as a consequence of changing agricultural practices and well-intentioned efforts to protect the habitat of the species. Unfortunately, these altered grazing regimes had drastic effects upon the lycaenid populations. The primary ant-species host (*Myrmica sabuleti*) could live only in fields closely cropped by livestock. Thus, even slight reductions in grazing allowed *Myrmica scabrinodis*, a low-quality host, to exclude *Myrmica sabuleti* from the area, thus leading to the butterfly's rapid local extinction (Thomas 1980; New 1991; Cushman & Murphy 1993).

Previous chapters and the discussion above reveal the critical importance of a demographic component - specialization. Clearly, the specialization that imbues both obligate mutualism as well as obligate aphytophagy in the Lycaenidae predisposes their practitioners to survivorship peril. This outcome, however, is not unique to the group - Kelley and Farrell (1998) suggest that based upon 'the law of the unspecialized' (Cope 1896), specialization may be an evolutionary dead end. They draw upon the phylogeny of host use in *Dendroctonus* (Coleoptera: Scolytidae) bark beetles to support their claim by revealing that specialists rose from generalists (in this case, at least six separate times), without reversal, all in derived positions, and closer examination of some specialists show instances where they appear to have lost particular host species from their diet. The generalist jack of all trades, master of none hypothesis is often invoked to explain the rise and prevalence of specialists (Via 1984; 1986; Simms & Rausher 1989; Tienderen 1991; Schulter 1995; Robinson et al. 1996). In this formulation, specialization is believed to have evolved as a consequence of trade-offs in performance of organisms on different hosts, such that optimized use of one host imposes limits on performance on others

(Simms & Rausher 1989; Futuyma & Moreno 1988; Jaenike 1990). While specialization is immediately attended by such advantages as freedom from natural enemies (Price et al. 1980; Atsatt 1981; Bernays & Graham 1988), long-term association with a particular host might eventually result in loss of genetic variation for the ability to use alternate hosts. Specialists might thus become irreversibly constrained on a particular resource or resources to the exclusion of others (Futuyma & Moreno 1988; Beissinger 1990; Via 1990; Siddal et al. 1993; Hougen-Eitzmann & Rausher 1994; Robinson et al. 1996). On account of such exclusionary use of limited resources, specialization is widely believed to result in a greater likelihood of extinction (Simpson 1953; Mayr 1963; Hansen 1980; Koch 1980).

The endangered Karner Blue (*Lycaeides melissa samuelis* Nabakov) is a prime example. Here, larvae feed exclusively on lupine (*Lupinus perennis*), an early successional species restricted to pine-barren habitats (Zaremba 1991). The existence of such habitats is extremely dependent upon intermittent fires. The anthropogenic suppression of fires and attendant habitat loss has hence significantly reduced the quantity and magnitude of the species' patchily distributed populations (Cushman & Murphy 1993). A complicating factor in specialization is that in a large percentage of cases for lycaenids, the species are myrmecophilous, which adds another level of analysis (please see previous discussion for *Maculinea arion*). Cushman and Murphy (1993) hence propose that butterfly species that associate with ants, particularly those species with strong dependence (presumably, obligately associated species) are far more sensitive to environmental changes and thus more prone to endangerment and extinction than species that are not tended by ants. Their contention is based on two factors – a) such species simultaneously require the right food plant and the presence of a particular ant species – a combination that is not frequent. These dual requirements likely result in spatial distributions, which are patchier than untended or sporadically tended species. b) selection may consequently favor reduced dispersal by myrmecophilous lycaenids, on account of the difficulty associated with locating patches that contain the appropriate combination of food plants and ants. The result may be that in addition to occurring as isolated populations, ant-tended species may express genetic traits associated with reduced outcrossing.

New (1993) also points out that increasing human recreational activities constitute another serious threat to many habitats all over the world, and he provides a few examples of the range of habitats involved. These include

- a) Coastal sand dunes in California that are threatened by off-road vehicles and trampling.
- b) Alpine heathlands and meadows in Europe and southeastern Australia are threatened by the construction of ski-lifts, runs, access roads, car parks, and resort accommodation and facilities.
- c) Pacific island habitats are threatened by the proliferation of golf courses and exotic vegetation.
- c) Mangrove swamps in eastern Australia are threatened by coastal resort development.

Conservation of Lycaenidae – the importance of surrogate species

Case studies of lycaenid conservation, or more generally, butterfly conservation are typically framed in terms of status reports. New (1996) suggests that such reports should incorporate the following imperatives:

- a) To demonstrate a conservation need, in response to a particular threat such as the development or destruction of a habitat patch.
- b) To designate priorities for allocating funds, or to defend preferential treatment for a particular taxon of conservation interest.
- c) To rank a series of habitat patches or sites in terms of their 'notable' species.
- d) To provide basic information on notable taxa as baseline information for pre-emptive use in countering anticipated threats.
- e) To provide a foundation for optimal management or recovery programs.
- f) To provide foundation data for long-term studies of geographical change
- g) To monitor the effects of long-term species, site or habitat management
- h) To monitor the fate of introductions or translocations.

These points are in part, an addition to, as well as an elaboration on, the recommendations of New (1993) for the conservation of any terrestrial group, which are as follows:

- a) Documentation and education to increase awareness at all levels and to facilitate communication between scientists in the field and the managers in charge of multiple land-use decisions.
- b) Detection and designation of habitats that support critical faunistic elements, be this a single species or suite of species that merit special conservation efforts.
- c) Investigation of interim legislative protection for particular taxa/habitats as additional documentation is procured.
- d) Autecological studies of selected taxa on which to base more wide-ranging management plans.
- e) Investigation of techniques for captive rearing.

The points raised by New (1993, 1996) all speak to the importance of particular species (and often, subspecies) or a small number of species, in terms of 'critical faunistic elements' 'notable taxa,' or selected taxa. These species are typically employed by conservation biologists as surrogates to help them address conservation problems (Thomas 1972; Panwar 1984; Bibby et al. 1992). There are various shades to the concept of 'surrogate species.' An 'indicator species' is that 'whose characteristics (e.g. presence or absence, population density, dispersion, reproductive success) are used as an index of attributes to measure for other species or environmental conditions of interest' (Landres et al. 1988). An 'umbrella species' is 'a target species so chosen that its minimum requirement area is at least as comprehensive as the rest of the community,' thus providing a protective umbrella (Wilcox 1984). Umbrella species differ from biodiversity indicator species in that they are used to specify the size and type of habitat to be protected rather than its location (Berger 1997). Flagship species are, by definition, employed to attract the attention of the public (Western 1987). Johnsingh and Joshua (1994) state that 'by focusing on one (flagship) species and its conservation needs, large areas of habitat can be managed not only for the species in question but for other less charismatic taxa.' This definition may be seen to be synonymous with that for an umbrella species and indeed, species can be both; however, the difference resides in that

flagship species only need to be popular, not necessarily of great ecological significance. Umbrella species, by contrast, need not be charismatic, and hence, not a flagship species. (Caro & O'Doherty 1999).

In summary, conservation biologists use surrogate species as a shortcut to monitor or solve conservation problems. 'Indicator species' have been used to assess the magnitude of anthropogenic disturbance, to monitor population trends in other species, and to locate regional biodiversity. 'Umbrella species' have been used to delineate the type of habitat or size of area for protection, and 'flagship species' have been employed to attract public attention. The terms are often used interchangeably, which can lead to erroneous conflation (Caro & O'Doherty 1999).

Important examples of surrogate species include the extinct Xerces Blue (*Glaucopsyche xerces*), the Karner Blue (*Lycaeides melissa samuelis*), the El Segundo Blue (*Euphilotes bernardino allyni*), and the Mission Blue (*Plebejus icariodes missionensis*), all from North America, the Brenton Blue (*Orachrysops niobe*) of South Africa, the Illidge's Ant-Blue (*Acrodipsas illidgei*), the Australian Hairstreak (*Pseudalmenus chlorinda*) and the Bathurst Copper (*Paralucia spinifera*), all from Australia, and the large blues (*Maculinea* spp.) and the Large Copper (*Lycaena dispar*) from most of the Palaearctic (New 1993).

There is little doubt that some charismatic lycaenid species designated as flagship species can do much to increase public awareness of conservation. However, such species-oriented conservation is essentially confined to the industrialized world, since conservation is largely considered a luxury in the developing world. One opportunity to promote significant species, however, is as 'local emblems' such as on postage stamps – lycaenids have been depicted on the postage stamps of nearly 70 countries (Coles 1991), which include both industrialized and developing nations.

The role of lycaenids as indicator species is relevant. Inasmuch as the local extinction of *Maculinea arion* was directly related to habitat change, the species can be viewed as much as an indicator species as a flagship. Hill and Sommer (1993) studied the Apollo Jewel (*Hypochrysops apollo*) in Australia to test its effectiveness as an indicator of habitat quality. Brown (1993), in his assessment of the conservation of neotropical lycaenids (which are still very poorly known) suggests that most are sporadically

distributed with great seasonal variation in presence and abundance. Such natural fluctuations lead to serious problems in establishing baseline data for lycaenids, or recognizing any significant tendencies that are consistent in different habitats. Attendant upon these problems is a difficulty in collecting and identifying species. These problems are compounded by the current state of uncertainty regarding the phylogenetic placement of several species, which diminishes the utility of neotropical lycaenids as ecological indicators (Brown 1993).

New (1997b) examines the issue of whether the Lepidoptera form an effective umbrella group whose conservation may assure or foster the well-being of others that can live in the same sites, and depend on similar suites of resources, without society necessarily obliged to devoting equivalent direct attention to the details of their conservation. With reference to lycaenids as umbrella species, in a survey of two Ontario sites for the Karner Blue (*Lycaeides melissa samuelis*), several rare Hymenoptera were detected. Even extinct forms can possess umbrella influence. The almost certainly extinct Palos Verdes blue (*Glaucopsyche lygdamus palosverdensis*) (Mattoni 1993) still serves to protect other species occurring in its vulnerable habitat because it still has not been officially delisted. The closely related lepidopteran family, Nymphalidae contains an important umbrella species earlier discussed, the Bay Checkerspot (*Euphydryas editha bayensis*) where Launer and Murphy (1994) showed that if all sites for the butterfly were preserved, up to 98% of the native plant species would also receive protection. Munguira et al. (1993), however, opine that policies concentrating on habitat conservation should be given priority over species-centered schemes although it is probably easier for policy makers to focus on the protection of a species rather than the more complicated process of habitat protection.

Three case studies

Having drawn attention to several examples in the literature, both lycaenid and otherwise, with emphasis on aphytophagy, I would like to speak of three case studies of lycaenid conservation that are currently under way with which I have some experience, either through personal communication or direct research. The first, *Orachrysops niobe*,

the Brenton Blue, is known only from a single locality in South Africa. *Cyclargus thomasi*, the Miami Blue, is restricted to a few islands in Florida, and the harvester, *Feniseca tarquinius*, one of the subjects of my own research, is found ephemerally and patchily in eastern North America (Opler & Krizek 1984). *C. thomasi* is phytophagous (Calhoun et al. 2002), *O. niobe* phytophagous and even potentially phytoredeous (Pringle et al. 1994) while *F. tarquinius* is an aphidophage (Riley 1886; Edwards 1886).

a) *Orachrysops niobe* (The Brenton Blue): Subsequent to the discovery of the species by Ronald Trimen in 1858 at Knysna, in what is now the Western Cape Province, South Africa, collection of the species was not reported in the literature until its rediscovery by Jonathan Ball at Nature's Valley, approximately 60 km east of Knysna (Pringle et al. 1994). Shortly thereafter, residential development destroyed the habitat and population of *O. niobe* at Nature's Valley. In 1991, Ball discovered a few *O. niobe* individuals flying at Brenton-on-Sea, close to Knysna, and the breeding grounds were soon discovered in the area by the amateur lepidopterist Ernest Pringle (Rand 1998). Since the Brenton at Sea site appeared to be the last breeding area known of *O. niobe*, the species came to be known by the colloquial name of the Brenton Blue.

In 1993, Knysna resident David Edge learnt that the Brenton-on-Sea habitat was marked for housing development. Together with Pringle, he campaigned with the local community, securing the help of members of the town council, the area hotel, and the regional leader of the conservation wildlife and environment society of South Africa, to initiate a fund-raising drive to purchase the Brenton Blue breeding grounds. In 1995, Edge commenced direct negotiations with the chief executive of the development project, Alan Rostovsky, arranging for a swap wherein a new road would be kept as far north as possible of the *O. niobe* habitat in return for which Edge forsook claims on a patch of habitat for a less endangered species in the area, the lycaenid *Chrysoritis mithras* (the Brenton Copper). The habitat of *O. niobe* was seen to cover 11 contested lots, and Rostovsky arranged for a moratorium on building until enough funds could be obtained for six, whereupon the other five would be swapped. Despite public exposure of the case through the local media, financial support was not immediately forthcoming, and it was only governmental intervention, through the ministry of environmental affairs and

tourism, that kept the habitat from falling into the hands of the developers. The governmental moratorium continues to be extended while options are being explored to raise the requisite funding. Most recently, the developer of the land has been paid its market value by the National Department of Environmental Affairs and Tourism, pending expropriation (Ernest Pringle personal communication).

Other complicating factors have included the discovery of another colony of *Orachrysops* at Kammanassie, far north of Brenton, which was at first considered to be *O. niobe*. Another breeding population would render the Brenton-on-Sea less significant and hence vulnerable to competing human needs. The Kammanassie population was eventually declared to be another species, and the Brenton-on-Sea population won a temporary reprieve. In all of this, the media has played a tremendous role in bringing the story of the Brenton Blue to the public, rendering it a flagship species for the Lycaenidae both in South Africa and internationally, in the face of development pressures (Rand 1998; Douglas Rand personal communication; Alan Heath personal communication). With governmental intervention, a 'management committee' including the Western Cape Nature Conservation Board, the Wildlife Society, the Lepidopterists' Society and the local Municipality have been treating the area as though it were a proclaimed reserve, with the establishment of an information center and the constant elimination of invasive plants (Ernest Pringle personal communication).

b) *Cyclargus thomasi bethunebakeri* (The Miami Blue): Much remains to be elucidated about the current viability status of the Miami Blue *Cyclargus thomasi bethunebakeri*, which renders it an appropriate flagship species for lycaenid conservation in Florida. Originally placed in the genus *Hemiargus*, the species and its congener *Hemiargus ammon* were both placed by Johnson and Balint (1995) in the genus *Cyclargus* as originally proposed by Nabakov in 1945. *Cyclargus thomasi* has five subspecies with the one in Florida, *C. t. bethunebakeri* bearing the trivial name, the Miami Blue. It was long suspected that the species in Florida was the closely related *C. ammon* (the Nickerbean Blue), although Calhoun et al. (2002) have, on the basis of actual specimens, shown that the species in question is actually *C. t. bethunebakeri*. *C. ammon* is a relatively recent arrival in Florida, with an original range restriction to Cuba, the Isle

of Pines and the Bahamas (Smith et al. 1994). It has since been found increasingly in Big Pine Key off the coast of Florida, though no other locations for the species have been found (Calhoun et al. 2002). The arrival of *C. ammon* in Florida has been coincident with the virtual decline of *C. t. bethunebakeri* although Calhoun et al. (2002) stress that this is not a cause and effect relationship, since the latter has been retracting southward for many years. In 1996, a colony was apparently extirpated through habitat restoration. Another population, however, was discovered in 1999 in the lower Florida Keys (the exact location has been undisclosed).

Documented hostplants for *C. t. bethunebakeri* include the native tropical trees and shrubs *Caesalpinia bonduc*, *Pithecellobium keyense*, and *Pithecellobium unguis-cati* (Matteson 1930; Brewer 1982; Lenczewski 1980). The development of coastal habitats in the region has led to a decline in the native hosts, and hence a corresponding decline in numbers of the insect. Numbers of a potentially alternative host, the exotic *Caesalpinia halicacabum*, which was locally abundant at the edge of tropical hammocks, also declined. The decline was exacerbated by the violence of Hurricane Andrew that devastated extreme southeastern Florida where a large proportion of the surviving populations of *C. t. bethunebakeri* were found. At another part of the range, the attenuation of numbers in the subspecies is probably due to another reason – in Big Pine Key, habitat loss does not appear to be the primary cause, pesticides may. Calhoun et al. (2002) suggest that *C. t. bethunebakeri* has been negatively affected by the widespread application, both aerial and terrestrial, of mosquito pesticides. Interestingly, *C. ammon* does not seem to be affected by application regimes, but Calhoun et al. (2002) argue that tolerance and adaptability to chemical exposure may vary widely between species. A number of causes may be hence attributed for the decline of *C. t. bethunebakeri*, but it appears likely that the relative isolation of the islands on which it is found, and the tremendous fragmentation of the species have prevented its rapid recovery (Calhoun et al. 2002).

All is not hopeless, however. The decline of the species is almost identical to *Eumaeus atala florida*, a once common lycaenid in southern Florida which declined precipitously through rapid urbanization and reduction of its native host, *Zamia pumila*. Captive breeding and translocation programs in appropriate habitats near Miami allowed for the

successful establishment of populations of *E. a. florida* (Emmel & Minno 1993; Hammer 1996), such that it is now even considered in some quarters a pest of cultivated cycads in the Miami area. *E. a. florida* is widely considered a success story for conservation among lycaenids, and it may be that similar translocation efforts may be undertaken for *C. t. bethunebakeri*, coupled with the controlled cultivation of its host-plants (Calhoun et al. 2002). In May 2001, the United States Fish and Wildlife Service (USFWS), on the basis of a petition, stated that the subspecies would receive emergency listing as federally endangered, if warranted. More comprehensive surveys are needed to determine the status of *C. t. bethunebakeri* in Florida, including all populations as far as possible, so as to effectively draft and bring into a play a sound recovery plan.

c) *Feniseca tarquinius* (The Harvester): *F. tarquinius* has already been discussed at length in Section 4 and this account will only address elements as pertains to its conservation. *F. tarquinius* has recently been considered for inclusion within the Massachusetts state wildlife list as a species of special concern (Jeffrey Boettner personal communication). Seldom common, there is little guarantee of finding the species in consecutive years at the same site. Much conservation concern resides in its peculiar life-habit that is aphytophagous, feeding only on species of aphids, most often, woolly aphids of the genus *Paraprociophilus* associated with speckled alder (*Alnus rugosa*) or beech (*Fagus grandiflora*). This specialization immediately renders the species dependent upon the host aphid, which, in turn is dependent upon a host plant, rendering the level of specialization particularly complex. Glassberg (2002) refers to *F. tarquinius* as rare or uncommon. Personal communication with several lepidopterists in the United States and Canada invariably has invariably elicited the same response – the species is hard to find. Widely distributed across Eastern North America, yet patchy and ephemeral, the species appears to find its great threat from habitat destruction (David Wright personal communication). On the other hand, it is also true that colonies may often be missed on account of lack of adequate sampling. Conservation strategies that could well be considered are the preservation of known habitats, the possibility of captive rearing (larvae have been raised to adulthood at constant greenhouse conditions at Harvard University) and phylogeographic approaches (Avise 1994; 2000) wherein population

structure can be tested to determine whether the species is marked by isolated demes or whether there is some measure of genetic interchange. The last is an especially useful tool when the species occupies a wide range, such as *F. tarquinius*.

SECTION 6

CONCLUSIONS

The path to aphytophagy in the Lepidoptera is rife with opportunity and strewn with peril. Weigman et al. (1993) frame the following question as the title of their paper, 'Diversification of carnivorous parasitic insects: extraordinary radiation or specialised dead end?' and it is pertinent to apply the question to aphytophagy in the Lepidoptera: The opportunity for ecological release or a route to an apparently dead-end? While the latter is surely in evidence, it is nevertheless intriguing and probably significant that the evolution of aphytophagy in the Lepidoptera has evolved multiple times in as many as eight superfamilies in the order (Cottrell 1984), and that despite its infinitesimally small representation (only about 215 documented cases in perhaps 200, 000 species), the phenomenon still persists. The subfamily Miletinae in the Lycaenidae represents one of the most spectacular radiations of aphytophagy in the Lepidoptera with included species running the gamut of feeding habit from active carnivory to trophallaxis (no miletine, however, has been known to be phytopredacious, unlike species of *Maculinea*).

Predatory lycaenid species appear to derive from a mutualistic template (Fukuda et al. 1978, Cottrell 1984), and their distribution is overwhelmingly Old World with particular concentration in the Afrotropical and Oriental regions. Hence, it is not surprising that the vast majority of aphytophagous lycaenids that depend directly upon ants are found in South East Asia, Australia, and Africa (again, the Palaearctic genus *Maculinea* is an exception). In the Miletinae, there are two patterns that emerge, one of direct dependence on the ant fauna (eg. active carnivory in *Liphyra*, *Allotinus*, *Miletus*, *Thestor*, or trophallaxis in *Euliphyra*, *Lachnocnema*, *Logania* and *Thestor*), or by contrast, active carnivory on Homoptera with typically little interference by the ants (eg. *Spalgis*, *Taraka*, *Feniseca*, *Logania*, *Allotinus*, *Miletus*, *Megalopalpus*). There is some measure of flexibility inherent in aphytophagy for some miletine species as seen from these examples, and Pierce et al. (unpublished) suggest that homopterophagy appears to be basal for most tribes in the Miletinae (with the exception of the Miletinae), with feeding on ant brood derived. In the basal Liphyrinae, myrmecophagy, trophallaxis and

homopterophagy are all manifested, which suggests that there may have been flexibility in aphytophagous dietary choice among ancestral miletines.

The constraints of ecology and phylogeny play out differently in various miletine groups underpinning on the one hand, the considerable radiation of the genus *Thestor* in southern Africa into over 25 species, for the most part in the relatively small region of the Western Cape of South Africa, and the lack of radiation of the Nearctic genus *Feniseca*, represented by only one species, *Feniseca tarquinius* over a land area that represents a considerable portion of half of the North American continent. Phylogenetic analysis of the species included in the genus *Thestor* indicate that the radiation may have occurred in a southern direction from a more central South African origin, with the most basal species, *T. basutus* found only in the northern provinces, and the vast majority of the most derived species located in the Western Cape. In all circumstances, the associated ant appears to belong to one species, *Anoplolepis custodiens*, which is intriguing (although Quek, unpublished, using molecular techniques, is examining whether the populations of *Anoplolepis custodiens* associated with different species of *Thestor* are in reality cryptic species). The morphological groupings of Heath and Pringle (unpublished) have been tested through the examination of DNA sequences obtained for the mitochondrial markers CO1 and ND5, and many of them have found support through the molecular analyses. Systematic analyses of different populations of *F. tarquinius* have as yet not been undertaken employing molecular methods, although there is no suggestion from the morphology that the populations indeed represent a suite of species, as opposed to a single one. The evanescent nature of *F. tarquinius* populations across its range, coupled with the complexity of its aphytophagous life-history renders it a prime candidate for conservation-based studies in the Lycaenidae, and potentially even a flagship species. Intriguing aspects of the biology of the species, including the length of the larval acoustic pulse train whose function remains unexplained, warrant further examination.

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