19. Amplified Species Diversity: A Case Study of an Australian Lycaenid Butterfly and its Attendant Ants

Naomi E. Pierce

Museum of Comparative Zoology Laboratories, Harvard University, Cambridge, Massachusetts

The family Lycaenidae comprises perhaps 40% of all butterfly species (Vane-Wright 1978). This remarkable success may result from their frequent association, in the larval stages, with ants (Chs 3, 6). Downey (1962*b*) noted that of 833 documented life histories of lycaenid butterflies, 245 species had myrmecophilous larvae (Colour plate 3B, C) illustrates two Australian examples). Both Hinton (1951) and Malicky (1969) argued that ancestral lycaenids were myrmecophilous, and in his recent classification of the group, Eliot (1973) also suggested that symbiosis with ants was an early development in the evolution of the Lycaenidae. In this paper, I will use the interaction between an Australian lycaenid, *Jalmenus evagoras*, and its attendant ant, *Iridomyrmex* sp. 25 (ANIC) (*anceps* group) as a case study to illustrate two possible ways in which ant/larval associations may have contributed to diversification within the Lycaenidae.

Specializations of Lycaenid Larvae

All the lycaenid caterpillars examined to date possess at least one adaptation that appears to be specialized for associating with ants (although not all lycaenids do associate with formicids; Ch.6). Studded over their surfaces are small epidermal glands called 'pore cupolas' that are thought to exude ant attractants, or 'appeasement' substances (after Hölldobler 1970). Malicky (1969) described in detail the histology and distribution of these glands, and reported finding them on the larvae and pupae of all 52 lycaenid species examined, but not on the riodinid, *Hamearis lucina*. More recent examination of *H. lucina* with the scanning electron microscope has shown that the larvae do in fact possess pore cupolas (Roger Kitching, pers. comm.). Epidermal extracts of two species have been bioassayed and shown to secrete substances that are attractive to ants (Pierce 1983).

Many species also possess one or both of two other ant-associated structures. The Newcomer's organ (or dorsal organ) is located on the dorsum between the seventh and eighth abdominal segments. In several species it has been demonstrated to secrete a mixture of simple sugars and amino acids (Maschwitz *et al.* 1975, Pierce 1983). It is flanked on either side by a pair of eversible tentacles that may secrete attractants to ensure the company of ants while the larva travels (Claassens & Dickson 1977), or act as defensive structures if the dorsal organ is depleted or the caterpillar is alarmed (Downey 1962*b*).

Jalmenus evagoras and Ants

Together with colleagues at Griffith University, I have been studying populations of *Jalmenus evagoras* that occur in Mt Nebo, Queensland. The range of *J. evagoras* extends from Melbourne, Victoria as far north as Gladstone, Queensland, in both inland and coastal localities (Common & Waterhouse 1981). It is characterized by dense aggregations of caterpillars that feed on many species of *Acacia*, and are tended by several species of *Iridomyrmex* ants. Both the late instar larvae (Pierce 1983) and the pupae stridulate when disturbed (Downey 1966; Ch.6); the vibrations may serve to alert attending ants. Pupation occurs on the foodplant, and clusters of pupae are also vigorously tended by ants (Colour plate 3C).

How do larvae of *J. evagoras* benefit from their relationship with ants? Malicky (1970) emphasized that by producing ant-appeasement substances, lycaenid larvae and pupae escape from ant predation. To examine whether or not tending ants also protect

larvae and pupae, we performed ant exclusion experiments (c.f. Pierce & Mead 1981) and found that tending ants are extremely effective at defending larvae and pupae against certain parasitoids and predators (Colour plate 3D). For example, when ants were removed, pupae suffered 95% parasitism by the chalcid wasp, *Brachymeria reginia*, whereas tended pupae were untouched. Differences in survivorship of tended versus untended larvae and pupae due to predation by wasps (*Polistes (Polistella) variabilis*), jumper ants (*Myrmecia nigrocincta*) and a variety of small spiders was even more pronounced (Pierce 1983).

We also determined that ovipositing females of *J. evagoras* (Colour plate 3E), (Atsatt 1981a) like several other lycaenids lay eggs preferentially on plants with ants. We positioned two groups of foodplants in the field so that one contained larvae with ants whereas the other had an equal number of larvae without ants. After maintaining this arrangement for four days, the control and experimental trees were exchanged and monitored for another four days to exclude any possible 'tree' or position effect. In both treatments, females laid overwhelmingly on those trees containing ants as well as larvae. When the experiment was repeated using the membracid, *Sextius virescens* to attract ants, the females again oviposited significantly more often on trees with membracids and ants (Pierce 1983).

Ant Rewards

What kinds of reward do ants receive for their protective role? Chemical analysis of the 'honeydew' shows that it consists of fructose, glucose, and sucrose in concentrations ranging from 5–55% during the course of the day, and high amounts of the amino acid serine, estimated at 50mM (Pierce 1983). In addition, analysis of the secretions from the surface of the caterpillars provides strong evidence that epidermal glands secrete concentrated amounts of amino acids. Several lines of evidence were used in this determination. First we analysed washings of both larvae and pupae in a Dionex® amino acid analyser equipped with fluorescence detection for high sensitivity, and found large amounts of serine, smaller quantities of histidine, glutamic acid, lysine, and arginine, and traces of aspartic acid, threonine, glycine, alanine, valine, isoleucine, leucine, phenylalanine and tryptophane. We replicated these amino acid 'profiles' for many individuals, and took care to assure that our samples were not contaminated by defecation, regurgitation, or residues left after moulting. Radioactive tracer experiments showed that labelled serine consumed by the larvae was rapidly passed to their attendant ants. We then used the amino acid profiles to concoct a 'soup' containing similar combinations of amino acids secreted by the

larvae and pupae. When we bioassayed this synthetic soup, it was found to be extremely attractive to workers of the attendant species, but of no interest to another species of ant (*Pheidole megacephala*) which does not favourably recognize the larvae and will attack them.

After these initial steps, an experiment was designed based on the observation that pupae of *J. evagoras* vary in their attractiveness to ants. We hypothesized that the attractiveness of pupae is directly related to the quantity of amino acids secreted by the pupae. To examine this possibility, an array of pupae was first monitored for ant attendance. These same pupae were then assayed for the amounts of amino acids secreted on their surfaces. There was a significant correlation between increasing attractiveness to ants and increasing amino acid concentration. Since the pupae do not possess a honeydew organ and can neither defecate nor regurgitate, we felt this was strong evidence that the amino acids in our samples were derived from epidermal glands only.

Finally, the chemical *o*-phthaldehyde was used to locate proteins and free amino acids on the caterpillars and pupae. The *o*-phthaldehyde, which we simply sprayed on the cuticle, combines with free amino acid groups and fluoresces under ultraviolet light. The dorsal organ, adjacent pore cupolas, and many of the modified setae located on the paired spines that run down the backs of the caterpillars, fluoresced with a purple hue that matched the colour emitted by serine treated with *o*-phthaldehyde (Pierce 1983).

Ants, Lycaenids and Nitrogen Rich Foodplants

We draw two main conclusions from these analyses. First, the ants tending *J. evagoras* may be receiving a significant nutritional reward in the form of amino acids as well as carbohydrates, the former being particularly important for the growth of ant larvae (e.g. Markin 1970, Brian 1956, 1973). The soup bioassays demonstrate that amino acids alone can serve as a strong phagostimulant for the ants, although it is quite likely that other compounds are secreted by the caterpillars and pupae to attract and appease ants. For those species of ants that are primarily nectar feeders and dependent on honeydew sources for their protein, caterpillars that secrete amino acids as well as carbohydrates could be an extremely important food resource (as suggested for extra-floral nectaries by Baker & Baker 1973a,b). Interestingly, the primary component of the lycaenid amino acid soup is serine, which is also a precursor of formic acid, and Gilmour (1965) remarks that it might therefore be of particular interest to formicine ants.

Second, these experiments indicate that while many

lycaenid caterpillars may benefit from ant attendance, they in turn must supply their associates with attractive rewards, and hence must feed on protein rich food sources. Indeed, the lycaenids are distinctive as a taxonomic group by their preference for nitrogen rich plant parts, such as flowers, seed pods, and terminal foliage (Mattson 1980). Many lycaenids feed on legumes (Ehrlich & Raven 1965), and a review of the species from Australia and South Africa shows a significant association between ant attendance and a preference for leguminous foodplants (Pierce 1983; but see also Ch.6). Moreover, some lycaenid larvae prey on homopterans, on ant larvae, and occasionally on each other (Hinton 1951).

Ant and Amplified Species Diversity Associations

Atsatt (1981*b*) discussed the adaptations that have evolved in the lycaenids as a consequence of associating with ants. He used the variables of ant abundance, ant predictability, and coincidence of ants with suitable foodplants to predict the nature of the likely interactions. What bearing might these different kinds of ant relationships have had on the evolution of diversity within the Lycaenidae? In the absence of a detailed fossil record providing unambiguous determinations of the relative ages of the butterfly families, I assume that the greater diversity of the Lycaenidae results from their relatively rapid speciation compared to other groups of butterflies rather than greater antiquity. I also assume that extinction rates of species do not differ greatly between the Lycaenidae and other butterfly families. Larval/ant associations may have influenced lycaenid speciation in at least two important ways.

Oviposition 'Mistakes'

The propensity of female lycaenids to use ants as ovipositional cues may have facilitated the process of host switching when the desired species of ant occurs on a novel foodplant (Atsatt 1981*a,b*). For example, during the course of a summer at Mt. Nebo, we observed *J. evagoras* switch onto four different species of *Acacia*, each of which was infested with membracids and *Iridomyrmex* sp. 25 before the switch occurred.

While few ovipositional 'mistakes' may have led to successful shifts, those changes would have been especially favoured in situations where the original foodplant was severely limited in occurrence within the range of the appropriate ant species (A 'mistake' refers to a case where a female oviposits on a plant that is not the usual larval foodplant and may or may not support larval growth; but see also Ch.7). In regions where ants are relatively scarce, there may have also been selection for ant generalists. However,

I agree with Atsatt (1981*b*) who proposes that once adaptations to ensure ant associations have been achieved, it may be more difficult to switch ant hosts than to switch foodplants. Within the range of their tending ants, hostplant switching may have occurred more easily for lycaenids whose protection relies on a mobile ant guard than for those species of butterflies that are dependent on specific toxins for defence (e.g. Brower & Brower 1964).

Ant association may have thus amplified the speciation rate of lycaenids beyond the usual hostplant-based level. An increase in the number of ovipositional mistakes would lead to an increase in the numbers of opportunities for subsequent speciation. Lycaenid females may make novel foodplant choices more often than females of other butterfly families because they select for ants as well as for chemically suitable foodplants. Even if we assume that the probability of these mistakes resulting in successful shifts is the same for lycaenids as it is for other taxa, the actual number of successes would be higher simply because lycaenids make mistakes more often.

Once a successful shift is achieved, speciation could occur in a number of different ways. For example, several authors, most notably Endler (1977) have indicated that it is theoretically possible for speciation to occur in the absence of geographic isolation. For ant attended lycaenids, this situation could be pictured most readily when the new hostplant occupies a different range from the original host. Although there is limited empirical evidence (e.g. Huettel & Bush 1972, Bush 1975, Guttman *et al.* 1981, but see Jaenike 1981, Futuyma & Mayer 1980) that changes in hostplant selection alone could result in speciation, such a possibility seems unlikely for lycaenids given the constraints necessary to ensure isolation between the original and derived populations. Finally, population isolates could form concomitantly with hostplant shifts if gravid females are blown or disperse far away from their source populations, and this could then lead to divergence amongst geographically isolated populations.

Population Structure and Speciation

The rate of proliferation within the Lycaenidae may have also been strongly influenced by the population structure of the butterflies. Field observations of *J. evagoras* in Australia and of another lycaenid, *Glaucopsyche lygdamus* (which I have been studying in Colorado), suggest that these butterflies occur in small semi-isolated demes. Both species are patchily distributed in areas that are often widely separated from one another. Individuals of both species, like many other lycaenids (Scott 1974*b*, 1975*d*, Gilbert 1979) are non-vagile, and accordingly there may be

little migrational interchange or gene flow between demes. This idea is supported by a mark-release-recapture study of *J. evagoras* in which we had extremely high recapture rates within an individual population (Pierce 1983). In addition, the size of these demes is often small. Both *G. lygdamus* and *J. evagoras* occur in quite limited areas, and in the case of *J. evagoras*, a deme is sometimes restricted to a single tree (Pierce 1983). Males of *J. evagoras* aggregate and compete for emerging females; clusters of as many as 15-20 males will surround a female pupa that is about to eclose (Colour plate 3F). Since not all males reproduce with equal success, the effective population size is thus further reduced.

Wright (1931, 1940) first showed that the structure and size of populations are important in determining the rate at which they evolve. Both he and subsequent workers (e.g. Mayr 1954, 1963, Lande 1976, Templeton 1980) have argued that the rate of evolution and speciation is much faster in species that have small and/or highly structured populations than in those that have large, panmictic populations. This argument has been used to explain the relatively rapid rate of speciation in placental mammals (Wilson *et al.* 1975, Bush *et al.* 1977), passerine birds (Baker, M. C. 1981), and herbaceous plants (Levin & Wilson 1976).

The patchy distribution and restricted size of populations of *J. evagoras* and *G. lygdamus* occur in spite of the availability of vast and continuous ranges of foodplants. I suspect that these range restrictions may result in part from selection for areas of foodplants that are both rich in nitrogen and coincident with tending ant species. Individual plants have been found to vary considerably in total nitrogen content, and in the 'quality' of nitrogen they produce (see Mattson 1980 for review). Many workers (e.g. Slansky & Feeny 1977, Morrow & Fox 1980, Auerbach & Strong 1981, Myers & Post 1981, Rauscher 1981a) have shown that such variation may have a strong impact on levels of herbivory. As previously mentioned, myrmecophilous Lycaenidae stand out because of their preference for protein rich foodplants and plant parts such as flowers and seed pods. This preference may carry over to discrimination between whole plants on the basis of their nitrogen content.

The predilection shown by lycaenids for nitrogen rich plants may be explained in part by the necessity of providing attendant ants with nutritional rewards in the form of amino acids. The distribution and size of lycaenid populations may have been restricted directly by the localized presence of potential attendant ants, and indirectly by the ants' requirements for nitrogenous rewards. By so doing, ant associations may have enhanced the rate of divergence of isolated or semi-isolated populations of butterflies, and hence their rate of speciation. In conclusion, the evidence gathered in studies of *J. evagoras* suggests two ways in which ants may have influenced the evolution of diversity within the Lycaenidae: first, by inducing a higher incidence of hostplant switching, and second, by modifying the population structure of the butterflies.