

## CHAPTER 18

# The Imperial Blue: *Jalmenus evagoras* (Lycaenidae)

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### ■ INTRODUCTION

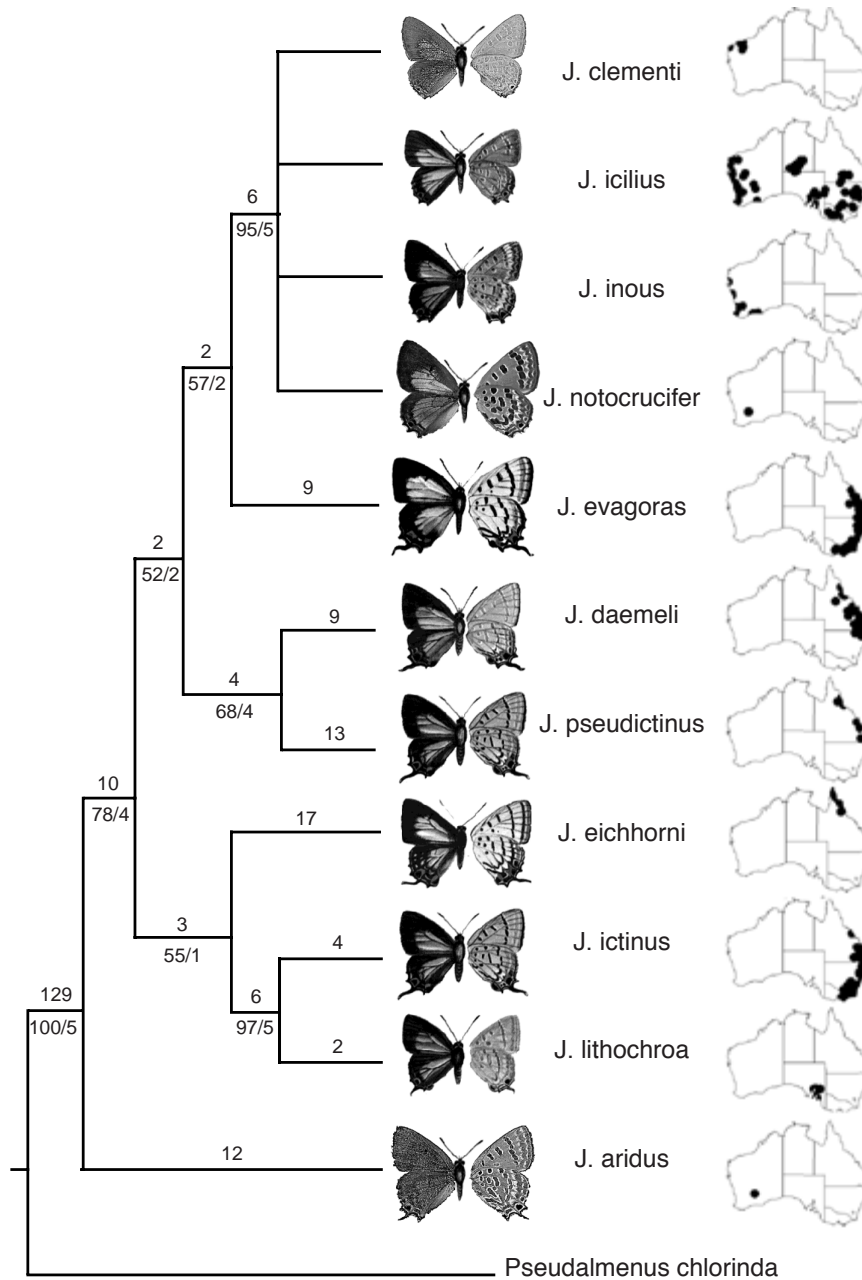
The interactions between the Australian lycaenid butterfly, *Jalmenus evagoras* Donovan, its *Acacia* host plants, attendant ants and suite of parasitoids and predators have provided a model system for the study of interspecific interactions, chemical communication, mutualism, and the evolution of complex life history traits. This review will describe the biology and natural history of this species, summarize research on its behavioural ecology and, where appropriate, indicate directions for future research.

The genus *Jalmenus* is endemic to Australia, and comprises at least eleven species (Fig. 18.1) whose life histories are well known in most cases (Common and Waterhouse 1981). The two most recently described species, *J. aridus* Graham and Moulds and *J. notocrucifer* Johnson, Hay and Bollam, are both from Western Australia and appear to be exceedingly rare; relatively little is known about their natural histories or distributions (Graham and Moulds 1988; Johnson *et al.* 1992). With the exception of *J. notocrucifer*, all species of *Jalmenus* have been recorded as feeding on *Acacia*. Although a number of species of *Jalmenus* have overlapping ranges and occur on similar host plants, they are commonly separated ecologically by their ant associations. For example, at a site near Kogan, Queensland, the adults of three species, *J. evagoras*, *J. ictinus* Hewitson and *J. daemeli* Semper, can be observed on the wing simultaneously, all within an area encompassing only about 1 sq. km. The larvae of all of these species feed on brigalow, *Acacia harpophylla* F. Muell. ex Benth., but the adults of each settle and lay eggs only on those individual plants populated by the appropriate species of attendant ant.

### Host Plants

The gregarious larvae of *J. evagoras* consume at least 25 different species of *Acacia* (Table 18.1). Eggs are laid in clusters, most often in crevices or holes in the bark, but occasionally on the leaves, stems, and in the hollow formed by the attachment of the leaf pedicel to the stem. The larvae pupate directly on the host plant, and pupae are also tended by ants. At locations in Queensland, ovipositing females often prefer the shorter, younger trees of *Acacia* (Smiley *et al.* 1988). However, juveniles are frequently found on considerably taller and older host plants in other locations (e.g. Braby 1988, 1998). It seems likely that the foraging behaviour of the attendant ant species influences the size of host plants chosen by females when they are laying eggs. In all cases, the foraging larvae feed preferentially on the terminal foliage of their host plants.

The numerous species of *Acacia* consumed by larvae of *J. evagoras* are not widely distributed throughout the genus, but are clustered phylogenetically. Although the systematics of *Acacia* is far from resolved (e.g. Brain and Maslin 1996; Playford *et al.* 1992), six of the host plant taxa listed in Table 18.1 fall within what has been recognised as the Racemosae group in the subgenus Phyllodinae, and nine are found in the Botryocephalae of the Bipinnatae (following the classification of Tindale and Roux, 1974). The significance of this phylogenetic association in host plant use in terms of the relative



**Figure 18.1** Species of the Australian genus *Jalmenus*, their geographic distributions and an estimate of their phylogenetic relationships. Distribution maps are based on information from Common and Waterhouse (1981) and Dunn and Dunn (1991). The phylogeny estimate is the strict consensus of three most parsimonious trees recovered by a branch and bound analysis (PAUP 3.1; Swofford 1993) of 2083 equally weighted, unordered nucleotides from the mitochondrial genome (1283 bp from *cytochrome oxidase* subunit I, 1728 bp from *cytochrome oxidase* subunit II, and 72 bp from the intervening tRNA leucine sequence). The number above each branch is the number of synapomorphies (steps) supporting that node. The first number below each branch is the bootstrap majority rule consensus value for 1000 bootstrap replicates of 10 random stepwise-addition heuristic searches per replicate (Felsenstein 1985); the second number below each branch is the decay index (Bremer 1988; Donoghue *et al.* 1992). Tree statistics (numbers in parentheses denote values for statistics calculated based upon informative characters only): length = 312 (208) steps, CI = 0.875 (0.812), RI = 0.819, RC = 0.717 (0.666). (Mignault 1996; A. Mignault and N.E. Pierce, unpublished data). (Butterfly wings scanned from dead specimens or from illustrations in Common and Waterhouse 1981; illustration assembled by C. W. Adams.)

TABLE 18.1. The recorded food plants of *Jalmenus evagoras*. Details of three newly recorded host plant species from Baylis (1989) and Nash (1989) are given as footnotes.

Acacia species	Reference
<i>A. binervata</i> DC.	Common and Waterhouse 1981
<i>A. dealbata</i> Link	Common and Waterhouse 1981
<i>A. decurrens</i> (H.L.Wendl.) Willd.	Common and Waterhouse 1981
<i>A. falcata</i> Willd.	Common and Waterhouse 1981
<i>A. filicifolia</i> Cheel & Welch ex Welch <sup>a</sup>	Baylis 1989, Costa et al. 1996
<i>A. fimbriata</i> Cunn. ex G. Don	Pierce and Elgar 1985
<i>A. floribunda</i> (Vent.) Willd. <sup>b</sup>	Nash 1989
<i>A. genistifolia</i> Link	Dunn 1984
<i>A. harpophylla</i> F.Muell. ex Benth. <sup>c</sup>	Common and Waterhouse 1981
<i>A. implexa</i> Benth.	Braby 1988
<i>A. ingramii</i> Tind.	Hawkeswood 1981
<i>A. irrorata</i> Sieber ex Spreng.	Common and Waterhouse 1981
<i>A. leiocalyx</i> (Domin) Pendley <sup>d</sup>	Hawkeswood 1981, Fraser (1997)
<i>A. leucoclada argentifolia</i> Tind. <sup>e</sup>	Nash 1989
<i>A. longifolia</i> (Andrews) Willd.	Crosby 1994
<i>A. macradenia</i> Benth.	Pierce and Elgar 1985
<i>A. mearnsii</i> De Wild.	Common and Waterhouse 1981
<i>A. melanoxydon</i> R. Br.	Common and Waterhouse 1981
<i>A. nerifolia</i> Cunn. ex Benth.	Common and Waterhouse 1981
<i>A. penninervis</i> Sieber ex DC.	Pierce and Elgar 1985
<i>A. polybotrya</i> Benth.	Common and Waterhouse 1981
<i>A. pycnatha</i> Benth.	Braby 1988
<i>A. rubida</i> Cunn.	Common and Waterhouse 1981
<i>A. spectabilis</i> Cunn. ex Benth.	Common and Waterhouse 1981
<i>A. terminalis</i> (Salisb.) Macbr.	Common and Waterhouse 1981

<sup>a,b</sup> Foodplants occupied by *J. evagoras* around Ebor, New South Wales;

<sup>c</sup> Foodplant of *J. evagoras eubulus*, not recorded for *J. evagoras evagoras*.

<sup>d</sup> Originally described as *A. cunninghamii* by Manski (1960: quoted in Hawkeswood 1981), which is a complex comprising at least six species. Hawkeswood (1981) suggested that *A. leiocalyx* was the most likely member of this complex. Ann Fraser (1997) has recently confirmed this species as a food plant on the campus of Griffith University, Queensland.

<sup>e</sup> A few trees were occupied by *J. evagoras* between Warwick and Stanthorpe, Queensland.

abundances of the plant species involved and/or aspects of their biochemistry or habitat distribution remains to be explored.

### Ant Associates

Attendant ants protect the juvenile stages of *J. evagoras* against predators and parasitoids, and in return, the larvae provide the ants with food rewards, secreted from specialized exocrine glands (Kitching 1983; Pierce 1983; Pierce *et al.* 1987). Although congeners such as *J. ictinus* and *J. pseudictinus* Kerr and Macqueen are highly species-specific in their ant associations in the sense that the larvae of each species are virtually always tended by a single species of ant, the larvae and pupae of *J. evagoras* associate with several different species of ants, primarily in the genus *Iridomyrmex*. At any one location, the juveniles are usually associated with one species; however, throughout the range of the butterfly, several different species of attendant ants are relatively common (Table 18.2).

Ants in the genus *Iridomyrmex* are dominant in all habitats within Australia (Greenslade 1985; Andersen 1995). Because of the large number of species, the wide geographic range of those species,

TABLE 18.2. Ant associates of *Jalmenus evagoras*. Based on data presented in Eastwood and Fraser (in review) and D. N. Merrill, M. Travassos, and N. E. Pierce (unpublished observations).

Ant species	Notes
<b>Tending under normal field conditions:</b>	
<i>Iridomyrmex anceps</i> group	The most common tending ant (e.g. Kitching 1983, Pierce <i>et al.</i> 1987). Differences in the morphology and ecology of <i>I. anceps</i> tending <i>J. evagoras</i> in different regions suggest that several species within the <i>I. anceps</i> species group may be involved.
<i>I. rufoniger</i> group	Another common tending ant (e.g. Pierce <i>et al.</i> 1987, Nash 1989 — referred to as <i>I. vicinus</i> ), often found in more arid areas. Again there are probably several species from the group that tend <i>J. evagoras</i> .
<i>Iridomyrmex</i> group containing <i>I. sucheri</i> and <i>I. mattiroloi</i>	A common tending species found at sites ranging from Victoria to Queensland.
<i>Iridomyrmex</i> sp. (aff. <i>gracilis</i> etcocles)	Collected tending <i>J. evagoras</i> on the campus of La Trobe University, Melbourne, Victoria (Braby 1989).
<i>Notoncus capitatus</i>	Found tending <i>J. evagoras</i> near Maryborough and Kogan, Queensland (Eastwood and Fraser, in review).
<b>Tending rarely or under unusual conditions:</b>	
<i>Iridomyrmex purpureus</i>	Found during a <i>J. evagoras</i> population explosion.
<i>I. agilis</i> group	Found tending a <i>J. evagoras</i> near Maryborough, Queensland.
<i>Papyrius nitidus</i>	Found during a <i>J. evagoras</i> population explosion.
<i>Camponotus</i> sp.	Found during a <i>J. evagoras</i> population explosion.

and the lack of phylogenetically informative morphological characters, the taxonomy of the group was problematic for many years (Greenslade 1979; Taylor 1987). However, S.O. Shattuck has recently greatly clarified the systematics of the Dolichoderinae and the genus *Iridomyrmex* in particular (1992a,b, 1993). Among the ant associates of *J. evagoras*, R.W. Taylor identified one of the most common tenders as belonging to the *I. anceps* species group, and they are referred to in this and in a number of other publications as *I. anceps* for simplicity. Similarly, another frequent tender found near Armidale, N.S.W., was initially considered to be in the *I. vicinus*–*I. rufoniger* species complex (see discussion in Nash 1989), and has been referred to as *I. vicinus* for simplicity in several publications (e.g. Costa *et al.* 1996). However, these ants have recently been re-examined by S.O. Shattuck, and found to be more consistent with the *I. rufoniger* species group. We will therefore refer to them as *I. rufoniger* here. Vouchers of all the ant associates of *J. evagoras* and its congeners are lodged at the Australian National Insect Collection (ANIC) in Canberra.

Associations with *I. anceps* and *I. rufoniger* account for perhaps 90% of the thousands of sightings of *J. evagoras* that these authors have made at field sites in Queensland and northern New South Wales during field seasons ranging from 1981 to 1996 (see also Eastwood and Fraser, *in review*). Populations in and around Canberra, A.C.T. and Melbourne, Victoria, have also been identified as commonly associating with members of the *I. anceps* species group. Finally, collections made by D.N. Merrill and M. Travassos from Queensland, New South Wales and Victoria have shown *J. evagoras* associating with an *Iridomyrmex* species group containing *I. sucheri* and *I. mattiroloi* (S.O. Shattuck, *pers. comm.*).

It is worth noting that in 1989, and again in 1991, populations of *J. evagoras* in northern New South Wales (near Armidale and Ebor) were unusually abundant, most likely because of early rainfall. In these years, the majority of the localized populations of *J. evagoras* were found associating with *I. anceps* or *I. rufoniger*, as in other years. However, in a surprising number of cases, one or two trees at any given field site contained juveniles of *J. evagoras* casually associating with several extremely different species of ants (Table 18.2). The situation seems to be analogous to an 'outbreak' year of a phytophagous insect, when a dramatic rise in the density of an herbivore results in the destruction of large stands of host plants (typically trees) and is often accompanied by the use of novel host taxa (Barbosa and Schultz 1987). This kind of outbreak phenomenon is significant because it suggests that the specificity seen in certain lycaenid–ant interactions may result in part from ecological forces such

as competition between ant species for lycaenids. Having said this, however, the clear phylogenetic association which can be seen between certain lycaenid taxa and their associated ants nevertheless indicates that historical processes and constraints presumably imposed by the difficulty of evolving complex chemical, morphological and behavioural cues to interact with novel ant species must also play an essential role in shaping species-specific interactions.

Colonies of *I. anceps* are typically relatively large and polydomous. When found in association with *J. evagoras*, the colonies frequently nest near the host plants, with nest entrances positioned at the base of each tree containing larvae of *J. evagoras*. *Iridomyrmex anceps* is predominantly monogynous: of the some 50 queenright colonies collected at sites in Queensland and northern New South Wales over the years, only two have been polygynous, one containing two queens and another three (N.E. Pierce, unpublished observations). Colonies of *I. rufoniger* are typically polygynous and polydomous, and when observed in association with *J. evagoras*, also tend to nest near the base of the host plant. Both ant species are generalist feeders, supplementing their diets with honeydew from homopterans, extrafloral nectar, elaiosomes, and dead insects.

### Range and Distribution

*Jalmenus evagoras* can be found along the east coast of mainland Australia, ranging from Melbourne in the south to just south of Gladstone in the north, and it is common along the coast as well as on the tablelands. The dependence of *J. evagoras* upon the overlapping requirements of appropriate species of both host plant and attendant ant accounts in part for its extremely patchy and localized distribution (Common and Waterhouse 1981; Smiley *et al.* 1988; see also Jordano *et al.* 1992; Costa *et al.* 1996). This patchiness is accompanied by strong site fidelity. For example, at one field site in Mt Nebo, Queensland, 74 out of 80 marked individuals were observed almost daily for their entire estimated adult lifespans ( $3.1 \pm 3.5$  days for females and  $6.9 \pm 5.9$  days for males; Mean  $\pm$  SD) (Elgar and Pierce 1988).

The species is multivoltine, with two broods commonly found in the south, and three or four in the north. Overwintering occurs in the egg stage. Males and females show little sexual dimorphism in wing colour or pattern. However, female pupae are larger than male pupae, and adult females can be as much as 60% heavier than adult males. For example, at the same field site in Mt Nebo described above, females weighed  $72.2 \pm 30.4$  mg (Mean  $\pm$  SD;  $N = 42$ ), whereas males weighed only  $45.7 \pm 17.3$  mg ( $N = 52$ ) (Elgar and Pierce 1988). The species is protandrous, with males eclosing several days before females. The sex ratio of individuals raised from eggs in the laboratory is 50:50 (N.E. Pierce, unpublished observations). However, because of protandry and the considerable difference in male and female survivorship, the effective sex ratio in the field is usually strongly male biased (Elgar and Pierce 1988; Costa *et al.* 1996).

### Subspecies

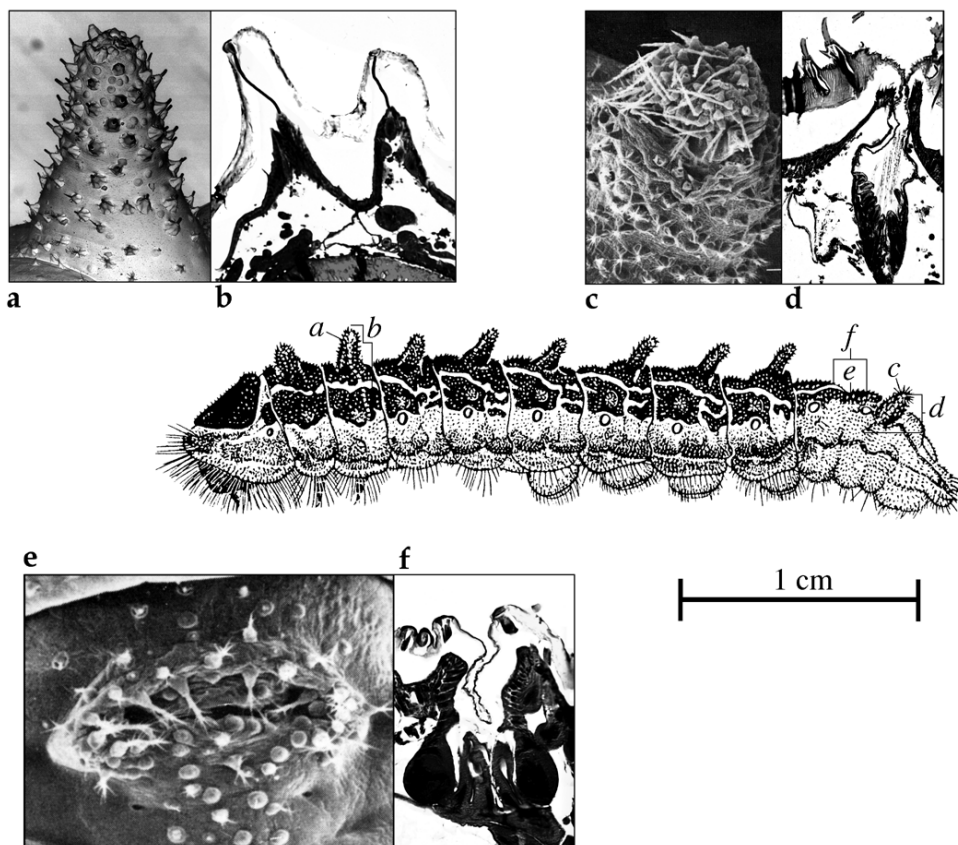
Two subspecies of *J. evagoras* are recognised: *J. evagoras evagoras* (Donovan) and *J. evagoras eubulus* Miskin (Common and Waterhouse 1981). The remainder of this chapter will be concerned entirely with the more common nominate species, which will be referred to as *J. evagoras* for brevity, and which has been studied in much greater detail than the highly localized *J. e. eubulus*. The biology of the two subspecies appear to be similar, with a few important differences. The most common foodplant of *J. e. eubulus* is brigalow (*Acacia harpophylla*) and the distribution of this subspecies seems to be restricted to the areas of arid brigalow scrub in South East Queensland and Northern New South Wales. The juvenile stages are similar to those of *J. e. evagoras* and have been found tended by the ants *Iridomyrmex anceps* and *Notoncus capitatus* (Eastwood and Fraser, *in review*), both of which also tend *J. e. evagoras*. Recent molecular phylogenetic studies (Taylor *et al.* 1993; Mignault 1996) support the subspecific status of *J. e. eubulus*.

## ■ MORPHOLOGY OF THE JUVENILE STAGES

### Ant-Associated Adaptations

The morphology of all the life stages of *J. evagoras* have been described in detail (Fig. 18.2) (Kitching 1976, 1983). As in other myrmecophilous lycaenids, the larvae and pupae of *J. evagoras* possess a number of specialized adaptations for associating with ants (reviewed in Cottrell 1984; also see Maschwitz *et al.* 1975; Henning 1987; Fiedler 1988; DeVries and Baker 1989; Fiedler and Maschwitz 1989; Ballmer and Pratt 1991; Hill 1993; Cushman *et al.* 1994; Axen *et al.* 1996). The larval cuticle is unusually thick, and contoured in such a way that the occasional nip or bite from an ant is unlikely to affect the vital organs (Malicky 1970). The larvae can range in color from green to almost black, and are cylindrical in shape, unlike many other lycaenid larvae which are onisciform. They possess pairs of fleshy dorsal tubercles that run down the back of the caterpillar from segments 1 to 6 and 8, as well as shorter lateral tubercles that protrude from the sides of segments 2 to 6.

Studded over the surface of both the larvae and the pupae are single-celled, epidermal glands called perforated cupola organs (PCOs), which are thought to secrete substances responsible for appeasing and perhaps rewarding attendant ants. On the dorsum of the seventh abdominal segment is the dorsal nectary organ (DNO), which secretes nutritious, sweet rewards for attendant ants. Like DNOs found



**Figure 18.2** Glandular anatomy of *J. evagoras* (fifth instar): (a) side view of dorsal tubercle studded with sensory organs and perforated cupola organs (PCOs); (b) sagittal section revealing glandular PCOs with openings at the surface of the dorsal tubercles; (c) tentacle organ (TO) partly extruded with bristles collapsing inward; (d) section of TO withdrawn into pocket showing glandular cells at base of bristles; (e) dorsal nectary organ (DNO) bearing branched setae near the entrance of the organ and studded with numerous PCOs; (f) section of DNO exhibiting secretory cells and PCOs. (Larva redrawn from illustration by S. Monteith in Kitching (1983); histological sections prepared by H. Engel and photographed by N. E. Pierce; SEMs photographed by N. E. Pierce and R. L. Kitching.)

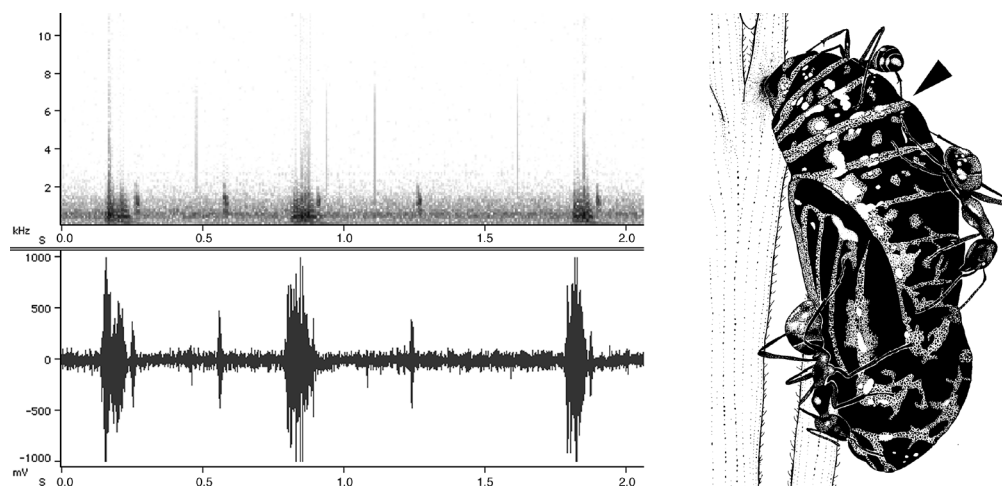
in other lycaenid larvae, this organ consists of four glandular cells that secrete into a central sac. The PCOs are not evenly distributed, but are concentrated near the DNO as well as on the tubercles, especially on the dorsum of thoracic segments 2 and 3 (Fig. 18.2). When the DNO has been appropriately palpated by an attendant ant, it puckers together and pushes upwards to extrude fluid from the central sac. Surrounding the DNO are numerous branched setae, which help to hold up the sugary droplets for the ants to imbibe, and which may play an additional, as yet uncharacterised, role in ant association.

Flanking the dorsal nectary on the eighth abdominal segment are the lateral tentacles, or tentacle organs (TOs). These glands are fully eversible and under the control of the larva. Their function is not fully understood, but they are thought to secrete volatile substances if a larva is alarmed, or if the DNO is depleted. The bristles at the tips of the TOs appear under the SEM to be without openings. Thus the volatile substances produced by the TOs may simply coat the surface of the bristles when the glands are retracted into their pockets, and be released when the organs are everted.

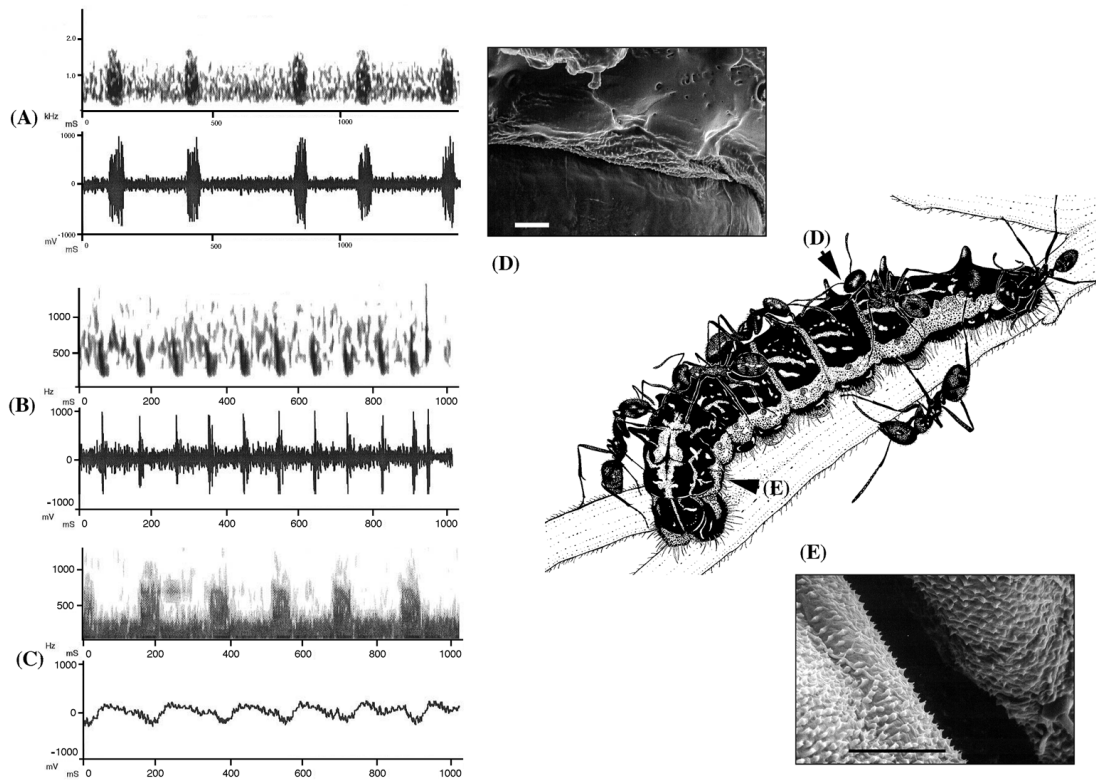
The histology of the larval glands has been examined for several of the larval instars, and cross sections of the different glands involved in the association show structures analogous to those observed in other species (Malicky 1969), including the glandular PCOs clustered near the dorsal organ and spotted along the tubercles running down the dorsum of the larva (Fig. 18.2).

In addition to organs secreting chemicals involved in promoting and maintaining ant associations, both the late instar larvae and the pupae possess stridulatory organs that are involved in communication with ants (Pierce *et al.* 1987; DeVries 1990; Travassos 1997). The pupae possess a file and plate stridulatory organ which is similar to pupal organs found in a number of other species (Downey 1966). A set of teeth on the anterior margin of the sixth abdominal segment (the file) is scraped against a series of ridges and depressions on the fifth abdominal segment (the plate). Travassos (1997) determined that the pupae produce two types of sounds, a primary and a secondary signal (Fig. 18.3). The primary signals have a higher amplitude than secondary signals and sound like a distinct 'chirp'. He noted that less well-developed sets of teeth are also found on the anterior portions of abdominal segments 5, 7 and 8, but in each case, these lacked an opposing plate.

The organs involved in larval calling are still not fully understood. However, Travassos (1997) found that late instar larvae have opposing sets of tiny teeth (one to seven microns in length) in a number of different intersegmental regions of the caterpillar. In contrast to the pupae, none of the teeth are opposed by a morphologically distinctive plate. Teeth oppose each other between the prothorax and



**Figure 18.3.** Sound production by pupae of *J. evagoras*. Shown on the left are the spectrogram (top) and waveform (bottom) of both the primary and secondary signals produced by the pupae. Illustrated on the right is an ant-tended pupa with an arrow indicating the position of the pupal stridulatory organ. (Redrawn from Travassos 1997; illustration of pupa by C. W. Adams.)



**Figure 18.4** Sound production by larvae of *J. evagoras*. Spectrograms (top) and waveforms (bottom) of three characteristic stridulatory signals are shown: (A) a grunt call, (B) a drum call, and (C) a hiss call. Scanning electron micrographs of possible stridulatory organs found on the margins of each abdominal segment: (D) above the spiracle line (see Fig. 18.2), teeth on the posterior margin of each abdominal segment oppose a smooth plate on the following segment; (E) below the spiracle line, each set of teeth is opposed by a second set of teeth. Arrows point to regions where photographs were taken, but note that each abdominal segment possesses similar organs. The scale bars represent 10  $\mu\text{m}$ . (Redrawn from Travassos 1997; illustration of larva by C. W. Adams.)

mesothorax, the mesothorax and metathorax, and the metathorax and first abdominal segment. On the abdomen, teeth are found on segments 1 through 8. These teeth are only found on one side (the posterior margin) of each abdominal segment on the dorsal region of the caterpillar above the spiracle line. In each case, the opposing margin is smooth (see Fig. 18.4D). However, below the spiracle line, along the ventral side of the caterpillar, teeth are densely distributed within the cuticular folds, such that each set of teeth is opposed by another set of teeth (see Fig. 18.4E).

Travassos (1997) demonstrated that the larvae of *J. evagoras* produce three kinds of calls: grunts, hisses and drums (Fig. 18.4 A,B,C). Knowing the widespread distribution of teeth in different intersegmental regions of the caterpillar, it seems likely that further research will show that the different calls are produced by particular sets of teeth in specific regions. Most importantly, Travassos also showed that different larval and pupal calls are emitted in different contexts: spontaneously, when disturbed, in the presence of conspecifics, and in the presence of attendant ants. The significance of these results for ant association will be discussed below.

#### ■ SECRETIONS

As described above, the larvae of *J. evagoras* possess at least three types of exocrine glands apparently involved in associations with ants: the PCOs, the DNO, and the TOs. To date, the secretions of only the DNO and the PCOs have been identified, and in these instances, for sugars and amino acids only.



### Tentacle Organs

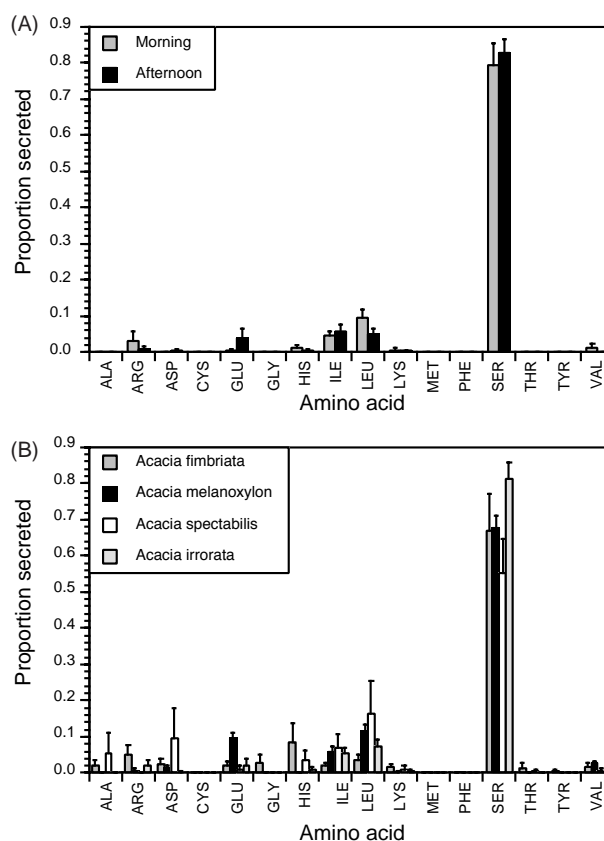
Secretions from the TOs remain largely uncharacterised. Despite repeated attempts to analyse volatile substances from the TOs, no compounds have been identified. Thus far, the only conclusion from these efforts is that the substances extruded by the TOs are not low carbon compounds, as previously suspected (T. Bellas, R. L. Kitching, and N. E. Pierce, unpublished). Indeed, the secretions from the TOs of another lycaenid species, *Shirozua jonasi* Janson have been reported to contain a high carbon compound, dendrolasin (Yamaguchi 1988). Whether similarities exist between secretions produced by different species remains to be investigated.

### Dorsal Nectary Organ

Roughly 10% by weight of the secretions from the DNO of *J. evagoras* consist of the sugars sucrose and fructose. They also contain significant amounts of free amino acids, particularly serine, at concentrations ranging diurnally from about 25 mM in the morning to 40 mM in the late afternoon. There are small, but consistent differences in composition of the DNO secretion according to larval host plant (Fig. 18.5) (Pierce 1983, 1989, and unpublished results).

### Perforated Cupola Organs

The pupae of *J. evagoras* possess glands that appear to be homologous with the larval PCOs, and seem to play a similar role in terms of appeasing and rewarding attendant ants. Secretions from the PCOs of the larvae and pupae also contain up to 14 different free amino acids, with serine being the primary



**Figure 18.5** The characteristic pattern of amino acids produced in the dorsal nectary organ secretions of *J. evagoras* (Mean  $\pm$  SE). (A) Secretions produced in the morning vary little from those produced in the late afternoon, although total concentrations are higher in the latter (see text). (B) The pattern of amino acids secreted varies slightly depending on differences in host plant species. The height of each bar represents the mean proportion of each amino acid relative to the total amino acid content of the secretion; error bars represent standard errors of the mean (N.E. Pierce, unpublished results; Pierce 1989).

component. Although we know the relative proportions of the amino acids in these secretions, it is not possible to measure the volume of the secretions produced, and thus we cannot determine their concentrations.

#### ■ EVIDENCE FOR MUTUALISM

The larvae and pupae of *J. evagoras* and their attendant ants provide a model system in which to tease apart the costs and benefits of the association for each interacting partner. Despite considerable discussion of the ecological importance of associations between ants and other insects, few studies have explored the exact nature of these interactions for both parties involved. Most interactions have been assumed to be mutualistic ones in which the action of each participant increases the fitness of its partner. A few studies have investigated the mechanisms by which ant-associated insects, or 'myrmecophiles', receive benefit from living with ants, but the reciprocal benefit to the ants in terms of food secreted by the myrmecophiles has been assumed without measurement. Thus an early goal of research into *J. evagoras* and ants was to measure costs and benefits of the interaction for both parties. In particular, these studies aimed to establish whether, under field conditions, the relationship between *J. evagoras* and its attendant ants was mutualistic or not.

#### Benefits for Butterflies

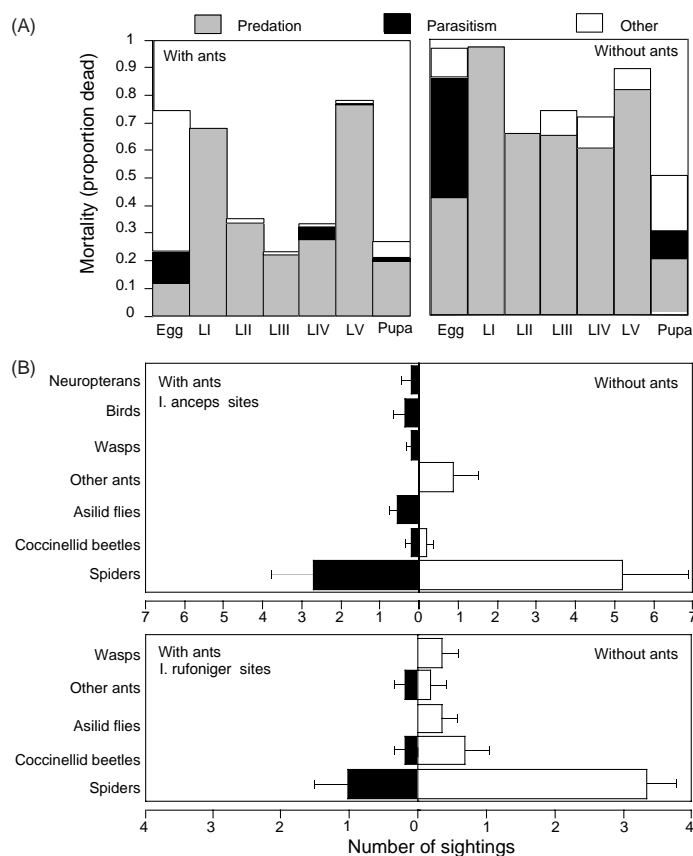
*Protection by ant guards.* A series of ant exclusion experiments were designed explicitly to examine the benefit that larvae and pupae of *J. evagoras* receive from their attendant ants. Field sites for three of these experiments were in or near Mt Nebo, Queensland, and a fourth on the outskirts of Canberra, A.C.T., in 1981 (Pierce *et al.* 1987); another was in Mt Nebo in 1986 (Baylis 1989; Nash 1989), and a third was in the Armidale–Ebor region of northern New South Wales in 1992 and 1993 (Wagner and Pierce, unpublished results). While the experiments in 1981 analysed survival of juveniles over a three-week period, the experiments in 1986 and 1992–93 encompassed the entire life span of an individual butterfly, from egg to eclosion as an adult (about six weeks).

The 1986 experiment, whose results are generally representative of those from all the experiments, showed that the survivorship for larvae on trees with ants was far greater than for those on trees where ants had been excluded: survivorship values were 1.83% for larvae with ants and none for larvae without ants (Nash 1989). In other words, parasitism and predation were so intense on trees without ants that larvae could not survive. On trees with ants, the vigilant guarding behaviour of the ants greatly reduces the presence of potential parasites and predators and provides a habitat in which survival is possible. Thus in a relative sense, trees patrolled by attendant ants can be regarded as an excellent example of what has been called 'enemy-free space' in the ecological literature (Atsatt 1981b).

A number of different agents contribute to the mortality of *J. evagoras* (Fig. 18.6). Cumulative observations of predators and their characteristic signs (sucked-out larval skins, carcasses encased in silk, etc.) indicate that most of the mortality when ants are excluded is due to predation by other arthropods, especially vespid wasps, predatory ponerine ants, reduviid bugs and spiders (Pierce *et al.* 1987; Nash 1989). Vertebrate predators, such as birds, although occasionally observed to take an adult, do not appear to contribute significantly to juvenile mortality.

The 1981 experiment failed to find a difference in survivorship of eggs on trees with or without ants (Pierce *et al.* 1987). However, subsequent experiments in both 1986 and 1993 demonstrated a striking difference in egg parasitism by a trichogrammatid wasp (*Trichogramma* sp.) on trees with and without ants (Fig. 18.7A), although the difference varied considerably from one field site to the next (Baylis 1989; Nash 1989; D. Wagner and N.E. Pierce, unpublished results). In both the 1981 and the 1986 experiments, ants provided considerable protection at the pupal stage against a chalcid wasp, *Brachymeria* (*Brachymeria*) *lasus* (Walker) (= *regina* (Giraud), that attacks the prepupa and ecloses from the pupa (Fig. 18.7C).

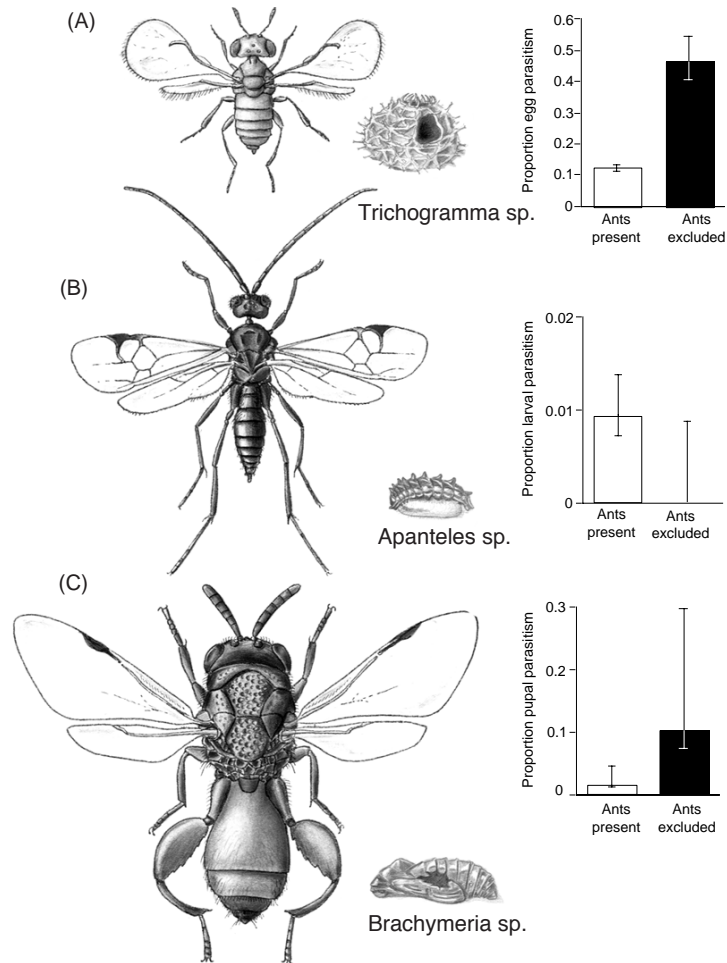
In an interesting twist to this general theme, however, one of the main larval parasitoids of *J. evagoras* is a braconid wasp, *Apanteles* sp., that attacks primarily early instars, and kills its host when it emerges from the fourth instar larva (Fig. 18.7B; e.g. Fiedler *et al.* 1995). Ant exclusion experiments



**Figure 18.6** Survivorship of juvenile stages of *J. evagoras* with and without ants. (A) The overall mortality of each instar during an exclusion experiment carried out in 1986 divided according to predation, parasitoid attack and unattributable mortality including disease and/or failure to moult. LI–LV represent larval instars 1 to 5. All larvae were originally tended by *Iridomyrmex anceps*, but these were excluded from *Acacia* trees bearing juvenile *J. evagoras* in the ‘without ant’ treatment (Baylis 1989; Nash 1989). (B) Observations of potential predators on *Acacia* trees bearing juvenile *J. evagoras* during an exclusion experiment carried out in 1992 and 1993, divided according to tending ant species. Bars represent the mean number of predators seen across six sites for each ant species; error bars represent standard errors of this mean (D. Wagner and N.E. Pierce, unpublished results).

failed to demonstrate any effect of ants against this parasitoid, and even indicated that parasitism was greater among larvae in the ant-tended group than among larvae in the group where ants had been experimentally excluded. Common and Waterhouse (1981, p. 497) specifically mention that populations of *J. evagoras* often suffer considerable parasitism by these wasps despite their ant attendants. The mystery was solved when a set of Y-tube choice experiments revealed that these wasps actually use ants as cues in finding their prey (Nash 1989). In a similar vein, Elgar and Pierce (unpublished results) found that orb-weaving spiders, which prey upon the larvae and adults, were more likely to colonize trees containing foragers of the attendant ant species than trees where ants had been experimentally excluded. Thus ant association in *J. evagoras* may carry a cost of increased apparency for certain specialized predators and parasitoids.

Finally, as a refinement of the all or nothing ant-exclusion technique used to measure the benefit to lycaenids, Robin Carper (1989; Pierce *et al.* 1991) designed a field experiment which provided a gradation of ant densities on host plants containing larvae of *J. evagoras*. Carper examined mortality rates of larvae on potted host plants of *A. decurrens* (*J. Wendl.*) Willd. positioned at increasingly greater distances from a large, central ant nest of *I. anceps*. In keeping with predictions from foraging theory, the farther the host plant was from the ant nest, the fewer attendant ants ventured out to tend

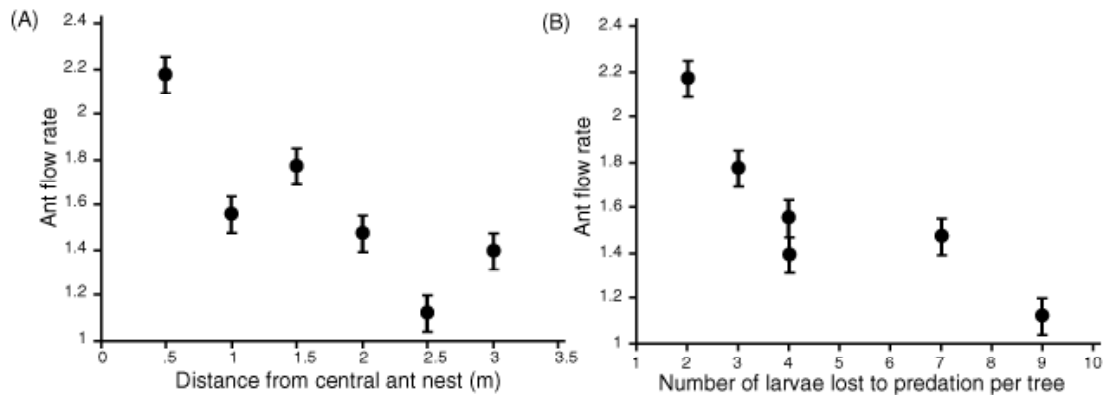


**Figure 18.7** The three most important parasitoids that attack *J. evagoras*: (A) a trichogrammatid wasp that attacks the egg, leaving a characteristic exit hole; (B) a braconid wasp that attacks the larva, emerging to pupate beneath the carcass; (C) a chalcid wasp that attacks the pupa, leaving a characteristic exit hole. Rates of parasitism by these parasitoids from the 1986 ant exclusion experiment are shown to the right of each figure. Bars represent the overall parasitism rate; error bars represent the 95% confidence interval of this estimate (Zar 1996). (Baylis 1989; Nash 1989; illustrations by D.R. Nash.)

the larvae (Fig. 18.8A). Carper's experiment demonstrated that this reduction in ant attendance (measured as ant 'flow' to the plants) led to increased predation rates (as measured by disappearance of larvae). Number of attendant ants per larva was negatively correlated with the probability of disappearance (Fig. 18.8B).

**Development: size and timing.** Since larvae of *J. evagoras* produce highly nutritious food rewards for their attendant ants, using material that would otherwise be available for their own development, it might be expected that ant-tended larvae can compensate for this by changes in either their feeding behaviour or their digestive efficiency. Two experiments were carried out to determine the effect of ant association on the development of *J. evagoras* larvae. The first focused on the effect that attendant ants may have on the entire juvenile period, from first instar until eclosion as an adult (Pierce *et al.* 1987). The second considered the effect of ants during the larval period only, and provided detailed measurements of digestive efficiency for the fifth larval instar (Baylis 1989; Baylis and Pierce 1992).

In the first, the general aim of the design was to simulate natural tending under field conditions by allowing ants to forage freely. Larvae were raised in the greenhouse on potted plants of *Acacia irrorata* Sieber ex Sprengel that were accessible to ants from captive, queenright colonies by means of stick

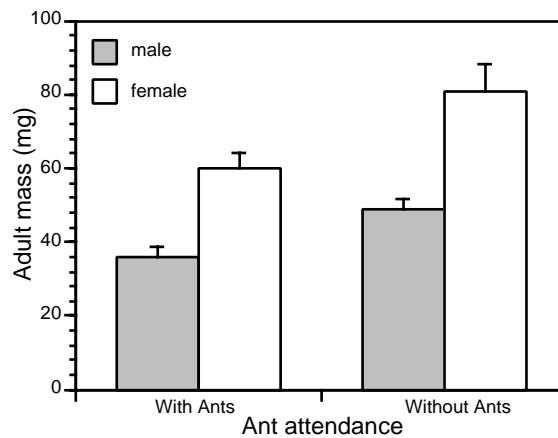


**Figure 18.8** Relationship between distance, flow rate of ants and survival of larvae of *J. evagoras*. (A) Variation in flow rates of ants to and from trees with larvae of *J. evagoras* as a function of foraging distance from a central ant nest (Mean  $\pm$  SE). Ant flow rate (transformed as the square root of the numbers of ants running to and from a tree per minute) was measured for potted host plants of *Acacia decurrens* containing six fifth-instar larvae of *J. evagoras* and placed at different distances from a nest of the ant, *Iridomyrmex anceps* (partial  $r = -0.365$ ;  $P < 0.0001$ ) (B) Predation as a function of flow rates of workers of *I. anceps* running to and from *A. decurrens* trees containing larvae of *J. evagoras*. The total number of larvae of *J. evagoras* lost from the trees described above over a two-week period are plotted as a function of ant flow rate to each tree (Kendall's  $T = -0.8$ ;  $P = 0.01$ ). Whenever a larva disappeared, it was replaced with another. (Redrawn from Carper 1989.)

bridges from the ant nests to the potted plants. Ants were allowed to tend larvae on one set of plants, but not on another, and plants were replaced with fresh plants every few days. The experiment demonstrated a strong effect of ant attendance on the development time of *J. evagoras*: larvae that were tended by ants developed almost five days faster than larvae that were not tended (out of a total juvenile period of approximately one month). This could be considered an added benefit of ant attendance inasmuch as the faster a larva develops, the less time it is exposed to potential predators. However, tended juveniles, particularly females, pupated at a significantly lower weight than untended individuals, and the adults that eclosed from these pupae were lighter and smaller (Fig. 18.9) (Pierce *et al.* 1987). The combination of faster developmental rate but smaller adult size meant that the growth rates of larvae reared with and without ants were not significantly different when considered over the entire juvenile period.

The second experiment necessitated close control and monitoring of factors influencing larval consumption. Larvae were reared individually on cuttings of *A. irrorata* in plastic test tubes, and a set number of workers from a colony of *I. anceps* was added and then removed from each of the test tubes of half of the larvae on a daily basis, while the other half remained untended but were manipulated in the same way. This experiment indicated that different instars of *J. evagoras* respond to the presence or absence of ants in different ways. Attendant ants again reduced the final size achieved by both males and females (Baylis 1989). However, ant presence or absence had little effect on the feeding rate or development time in the fifth larval instar, and digestive efficiency was actually lower when tended. Fifth instar larvae in the tended group did not appear to compensate in any way for the material lost to their attendant ants. The result was that they converted a smaller proportion of ingested food into their own biomass, and consequently grew less than their counterparts without ants (Baylis and Pierce 1992).

Interestingly, males and females differed in their relative growth rates during this period, with males converting a larger amount of food more efficiently into biomass than their female counterparts. Males also pupated sooner than females. However, in the transition from pupa to adult, males lost on average  $60.4 \pm 1.2\%$  of their pupal weight, whereas females lost significantly less of their pupal weight and were remarkably more variable in this response ( $35.2 \pm 6.5\%$ ,  $F_{1,52} = 364$ ,  $P < 0.001$ ; Chang 1988). This was true whether individuals were raised with or without ants. The significance of these kinds of developmental differences between the sexes remains to be fully explored.



**Figure 18.9** The mass of adult males and females of *J. evagoras* when reared with or without attendant ants in the laboratory. The height of each bar represents the mean mass of each sex ( $\pm$  SE) under each condition. (Redrawn from Pierce *et al.* 1987.)

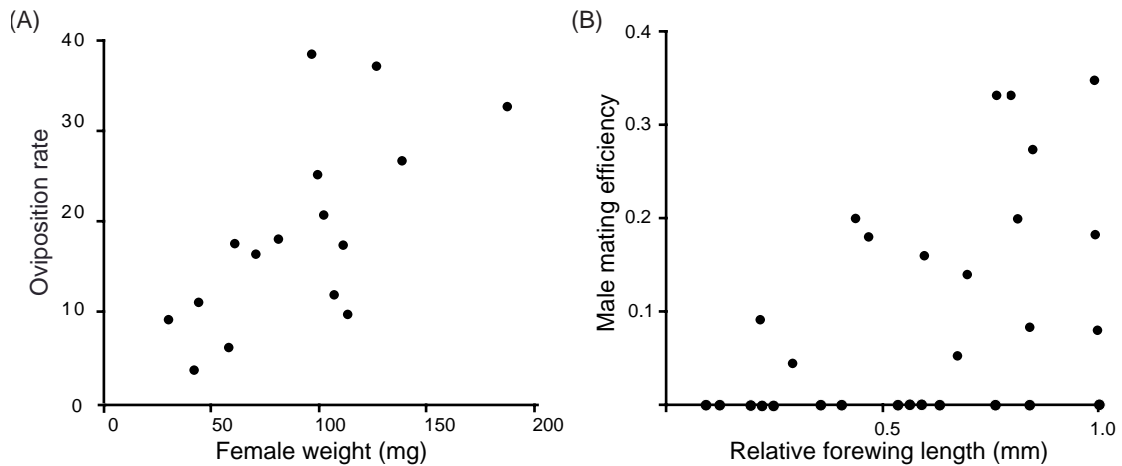
Additional research has documented that behaviours other than feeding can be influenced by larval age (Pierce *et al.* 1987; Baylis 1989; Nash 1989). For example, the first three larval instars are more likely to aggregate when they are tended by ants than when they are not, whereas the behaviour of the fourth and fifth instars is not changed by the presence or absence of ants. Further research is necessary to clarify the effects that attendant ants have on development of each larval stage for both sexes of *J. evagoras*. It is particularly striking that the presence or absence of attendant ants can influence a larva's decision about when to pupate. The mechanistic basis of this sensitivity remains to be understood (but see Nijhout 1975, 1981). Further research might reveal whether an external cue such as ant attendance can alter the timing of the onset of metamorphosis, or whether ant attendance alters internal cues as a byproduct of the provision of rewards for ants.

### Costs for Butterflies

**Size and lifetime reproductive success.** Larvae and pupae of *J. evagoras* produce metabolically expensive secretions to feed attendant ants and, as noted above, the costs incurred by this secretion are reflected primarily by a reduction in adult size. To examine whether a reduction in adult size represents a true cost for *J. evagoras*, each sex must be considered separately.

For females, reduced size is associated with a reduction in fecundity (Fig. 18.10A). Females of *J. evagoras* emerge with a number of mature eggs already present in the oothecae, and the number of these eggs is positively correlated with the weight of the butterfly on emergence (Hill and Pierce 1989). The number of eggs laid by a female over her entire lifetime is also positively correlated with her weight at emergence (Hill and Pierce 1989). Thus small size appears to be a cost of ant attendance for females of *J. evagoras*. However, this relationship is not entirely straightforward. Although they may be smaller on average, for reasons that we do not yet understand, the realised fecundity of females that have been tended by ants is significantly greater than that of their same-sized counterparts that have not been tended (Chang 1988). In other words, when the effects of size have been taken into account, ant-tended females lay more eggs than non-tended females. Thus small size may not represent as great a fecundity cost as would appear on first glance.

A further consideration is that while ant-tended females of *J. evagoras* pupate at a smaller size, they also pupate and eclose sooner than their untended counterparts. This could potentially give a competitive advantage to ant-tended females in the search for oviposition sites. In the northern part of its range where *J. evagoras* regularly goes through an indeterminate number of broods, those populations whose development is hastened by attendant ants could perhaps add an additional brood in a season. This might counteract any deleterious effects of small size, and possibly even act as a net benefit of ant attendance. This, however, remains conjectural: we know little as yet about the



**Figure 18.10** The effect of adult size on components of fitness in *J. evagoras*. (A) The relationship between the mass of a female of *J. evagoras* and her oviposition rate reared under laboratory conditions ( $r_s = 0.641$ ,  $P = 0.004$ ). Oviposition rate is the total number of eggs laid per total number of days alive. (B) The relationship between male size (relative forewing length) and mating efficiency ( $r_s = 0.475$ ,  $P < 0.005$ ). Mating efficiency is the number of realised mates per total number of potential female mates that eclosed during a male's lifetime. (Redrawn from Elgar and Pierce 1988.)

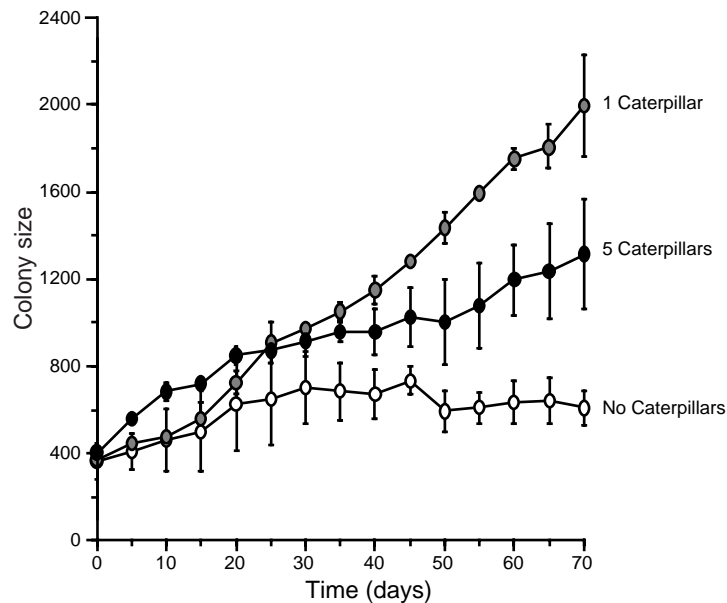
phenology of the species in different localities, or how it may be influenced by location-specific ecological factors, such as the availability of different host plant species, or ant associates.

For males, size is a critical determinant in lifetime mating success (Elgar and Pierce 1988). Males of *J. evagoras* engage in active contests for eclosing females. Because of the visible and predictable location of pupae, males are able to search systematically for prospective mates. Shortly before eclosion, a pupa secretes a volatile chemical that attracts males. Dozens of males may surround a pupa, and an emerging female is often mated before she has even expanded her wings. In a study of a population of marked individuals, Elgar and Pierce (1988) determined that there was high variance in male mating success, and that the relative size of a male was correlated with his mating efficiency, or the number of mates he had out of the total number of females that eclosed during his lifetime (Fig. 18.10B). Small size thus represents a cost to males of *J. evagoras* in terms of lifetime mating success.

Once again, however, the effect of ant association can have complex effects. Like many other butterflies, *J. evagoras* is protandrous: males emerge before females, and the effective sex ratio in the field is strongly male biased. Elgar and Pierce (1988) found that males eclosing earlier in the season had more opportunities for mating than males emerging later in the season. Presumably there is a trade-off in development among males between time of emergence and adult size: the early-emerging males sacrifice size for opportunity, while the late emerging males forego opportunity in favor of larger, more competitive size.

### Benefits for Attendant Ants

**Food rewards.** For attendant ants, the benefits of associating with juveniles of *J. evagoras* are reaped as food rewards from larval secretions. Two approaches have been taken to measuring these benefits, one to measure the gain in biomass from harvesting larval secretions over a set period of time (Pierce *et al.* 1987), and the second to determine whether harvesting secretions is translated into an increase in ant colony growth (Nash 1989). Ants foraging on a single tree of *A. irrorata* in the field containing 62 juveniles of *J. evagoras* collected approximately 400 mg dry weight of food over a 24 hour period. Given that an average worker of the attendant ant species, *I. anceps*, weighs only about 0.4 mg (dry weight), such a resource could constitute a considerable proportion of the overall food budget of an attendant ant colony (Pierce *et al.* 1987).



**Figure 18.11** Growth of laboratory colonies of *Iridomyrmex rufoniger* when allowed to forage from different numbers of larvae of *J. evagoras*. Each point represents the mean ( $\pm$  SE) combined number of larval, pupal, and worker ants in two or three replicate colonies. The graph illustrates the growth of colonies after eggs laid during the experiment had started to hatch (before this period there was a decline in the size of all colonies since no new larvae were being recruited into the colonies). (Redrawn from Nash 1989.)

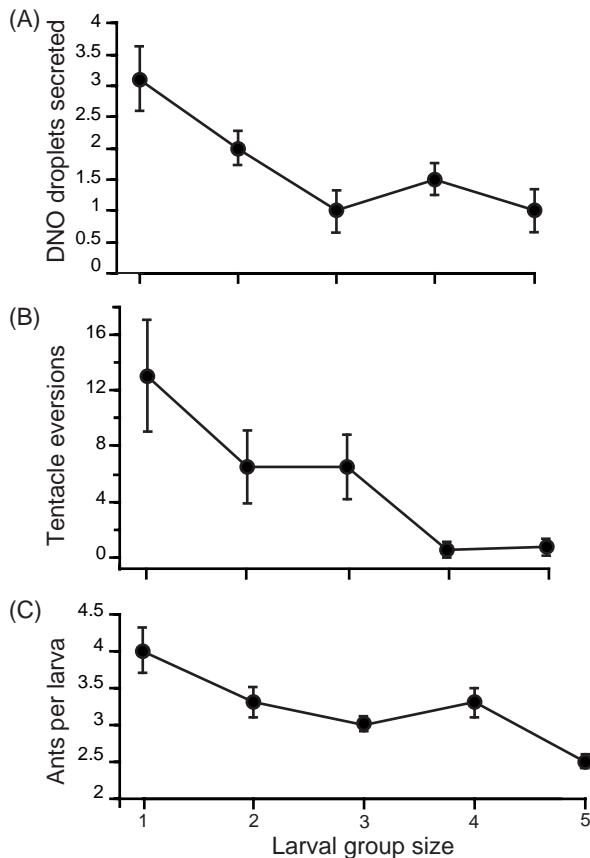
However, measuring rates of food uptake does not provide information about the effect of that food on colony growth. Colony growth is a better measure of the benefit received by the attendant ants, inasmuch as it takes into account the metabolic costs of foraging for, defending, and digesting that food. Because its size is correlated with the number of reproductives a colony can produce, colony growth reflects the effect of lycaenid food secretions on the fitness of their ant partners. Nash (1989) raised colonies of an attendant ant species, *I. rufoniger* group, on three food treatments: workers were allowed to forage on *A. decurrens* trees containing no larvae, one larva, and five larvae over a 120 day period. The secretions obtained from either the lycaenids or the extra-floral nectar of the host plants were supplemented by unlimited quantities of an artificial ant diet (Bhatkar and Whitcomb 1970, modified as per Nash 1989).

This experiment yielded a fascinating, but puzzling result. In all cases, colonies had higher growth rates when allowed to forage on secretions from larvae of *J. evagoras*, showing that the relationship between *I. rufoniger* and *J. evagoras* is truly mutualistic. However, the colonies allowed to forage on the secretions of only one caterpillar grew significantly more than those allowed to forage on the secretions of five caterpillars (Fig. 18.11).

A number of alternative hypotheses could account for this unexpected observation, and more research is needed to resolve the issue. A mundane possibility that cannot be discounted relates to limitations of the experimental design, since the physiological effect of five larvae defoliating a small potted host plant is likely to be qualitatively different from the effect of a single larva. Although an attempt was made to minimise this effect by rotating the food plants between the treatments, it is still possible that the result reflects an interaction between the host plant and the level of herbivory rather than something intrinsic to the larvae themselves. To control for such effects in the laboratory, larvae should ideally be raised on cuttings from the same host plants. Attempts to repeat ant colony growth experiments using this technique have thus far failed because of increased larval and ant mortality, possibly in part due to the increased stress caused by such manipulations.

It is likely that lycaenid larvae adjust their secretion rates depending upon their social context. Larvae of *J. evagoras* aggregate under natural circumstances, and as described earlier, are more likely to do so





**Figure 18.12** Response by 4th instar larvae ( $N = 20$ ) of *J. evagoras* to changes in group size. The graphs plot mean observations ( $\pm$  SE) from focal individuals over a 5 minute interval for (A) secreted food droplets, (B) tentacle eversions, and (C) per capita attendance by ants allowed free access to the larvae. Each larva experienced all five treatments. (Redrawn from Axén and Pierce 1998.)

in the presence of ants. The mean number of attendant ants per larva tends to increase with age, and larger larval groups attract a greater total number of ants, even though the *per capita* numbers of ants may be less (Pierce *et al.* 1987; Nash 1989). Secretion of rewards for ants is developmentally costly, and thus selection may favour aggregation if individuals in groups can secrete less but still recruit a sufficient number of ant guards. In other words, the cost-benefit ratio for individual larvae would be expected to change with changing group size.

Axén and Pierce (1998) tested this hypothesis by examining secretion rates of larvae in different sized groups, and found that secretion rate decreased with increasing group size. When aggregated, larvae provided fewer food rewards than when solitary, whether the number of attendant ants was held constant or allowed to vary freely (Fig. 18.12). This could partially account for the unexpected results seen in the ant colony growth experiment, since caterpillars in groups of five produce less secretion *per capita* than solitary caterpillars. However, the overall quantity of secretion produced by five caterpillars should still be greater than or at least equal to that produced by a single caterpillar.

A final explanation is that the costs and benefits of harvesting secretions do not change in a linear fashion with the number of caterpillars, so that the rewards reaped from five caterpillars does not compensate for the cost of harvesting those rewards. From this point of view it is interesting to note that although Axén and Pierce (1998) found a decrease in both the rate of secretion by larvae and the per capita level of ant attendance with increasing larval aggregation size, the relative decrease in ant attendance (which is presumably related to the costs involved — see below) was not commensurate with the relative decrease in secretion rate.

### Costs for Ants

**Metabolic Costs.** The experiment examining the growth of ant colonies described above actually subsumes metabolic costs into the equation, because colony growth is a net measure incorporating

both costs and benefits. In addition, the energetic cost of foraging by *I. anceps* on pupae of *J. evagoras* has been investigated using respirometry. These measurements indicated that the metabolic cost of processing lycaenid secretions was large relative to the estimated gain from the secretions (Nash 1989). In this analysis, however, pupae measured without ants were not 'silenced', and thus one reason why the estimated gain appeared to be so low could have been because pupae deprived of ants expended less energy stridulating than their tended counterparts. Nevertheless, the relatively small returns raised questions as to how ant colonies decide how to allocate foraging between lycaenids and potentially competing mutualists such as homopterans (Nash 1989). The secretions of *J. evagoras* contain considerably greater concentrations of amino acids than the secretions of most homopterans (see later discussion), and thus differ qualitatively as food rewards. Whether this difference alone can account for the extreme attractiveness of juveniles of *J. evagoras* to their attendant ants is not known. A full answer to this question requires a more thorough understanding of the composition of the secretions, as well as the nutritional requirements of the ants.

*Opportunity costs.* If tending lycaenid larvae occurs at the expense of tending alternative, more nutritious food sources (such as other trophobiotic insects for example, aphids or coccids), then the relationship might sometimes incur opportunity costs for the ants. Such a scenario could give rise to the evolution of parasitism if it were less expensive metabolically for the lycaenids to resort to fooling ants chemically rather than rewarding them. Indeed, parasitic species of lycaenids are known in which the larvae are carried by their associated ants into the nest where, chemically camouflaged, the lycaenids devour the ant brood (Cottrell 1984; Thomas *et al.* 1989). Moreover, such instances of parasitism have evolved more than once within the Lycaenidae. All the species in genera such as *Maculinea*, *Acrodipsas* and *Lepidochrysops* appear to be parasitic and/or aphytophagous; other examples can be found of predominantly phytophagous genera where only one or two species have become parasitic, including *Arhopala*, *Ogyris* and *Chrysoiritis* (Pierce 1995; M. Braby *pers. comm.*; A. Heath *pers. comm.*).

David Merrill (1997) explored the possibility that larvae of *J. evagoras* can manipulate ant behaviour more explicitly by examining components of the growth and survival of artificially created colony fragments generated from four queenright colonies of *I. anceps* collected at Mt. Nebo. Merrill divided these colonies into queenless fragments, or 'nests', which consisted of 15, 35 or 80 workers respectively, each supplied with 2 mg of eggs (approximately 70 eggs) which had been extracted from their respective colonies of origin. Two nests of each size were removed from each of the four queenright colonies, one which served as the control, and the other the experimental treatment. Both treatments were provided access to unlimited quantities of ant diet (Bhatkar and Whitcomb 1970); however, experimental treatments were allowed to tend one late instar larva of *J. evagoras*, whereas controls were not.

Using this method, Merrill demonstrated that in small nests, where the ratio between adults and brood was sufficiently low that workers were faced with the decision to tend either the caterpillar of *J. evagoras* or their own brood, the ants preferred to tend the caterpillar, and let their own brood perish. This finding was consistent with those of Evesham (1985) and MacKay (1985) who found for four different species of ants that the number of workers caring for the brood and not the amount of available food was the most important determinant of brood survivorship. The significance of Merrill's results is that under certain circumstances, the mutualism between *J. evagoras* and its associated ants can turn to parasitism, with the lycaenid larvae manipulating the attendant behaviour of their associated ants.

To summarize, experiments measuring proximate costs and benefits in the association between *J. evagoras* and its attendant ants have raised as many questions as they have answered. Nevertheless, they demonstrate that the relationship between the two partners is (1) mutualistic, and (2) essential for the butterflies. The benefits of ant attendance to larvae of *J. evagoras* are greater survival from attacks by predators and parasitoids, and a faster and qualitatively different developmental rate. The cost to *J. evagoras* of depending on ant guards is meted out metabolically, and reflected in smaller adult size, which can have a deleterious effect on the lifetime reproductive success of both females and males.

Moreover, because of its obligate association with ants, juveniles of *J. evagoras* are also subject to attacks from specialised enemies such as parasitic wasps and predaceous spiders that use ants as cues in finding their hosts.

For the ants, the prime benefit of associating with *J. evagoras* is nutritional, although the nature of those nutrients and their role in the overall nutritional ecology and population dynamics of the ants remain poorly understood. We must learn more about the quality of the nutrients themselves in order to ascertain whether the relationship is consistently mutualistic, or whether the lycaenids sometimes manipulate their attendant ants into providing protection without appropriate rewards. In some cases, the ants may suffer opportunity costs if they devote their efforts to tending lycaenids rather than seeking out alternative food resources, or if they neglect their own brood by diverting attention to grooming and caring for lycaenids. In general, however, the main cost to the ants appears to be the metabolic cost involved in finding, defending, foraging on and digesting the larval secretions.

#### ■ FACTORS AFFECTING THE NUMBERS OF ANTS TENDING LARVAE AND PUPAE

Several factors are known to influence the numbers of ants attracted to individual larvae and pupae (see discussion in Pierce *et al.* 1991). On the one hand, levels of attendant ants are presumably shaped through the quantity and/or quality of the secretions provided by the larvae, and factors such as host plant quality and larval size, age and social context can all affect the production of these secretions. On the other, factors influencing the ants themselves, such as their distance to the larvae (discussed earlier), their access to alternative resources (i.e. their dietary history), and their colony size can all influence the numbers of workers sent out by the colony to the larvae.

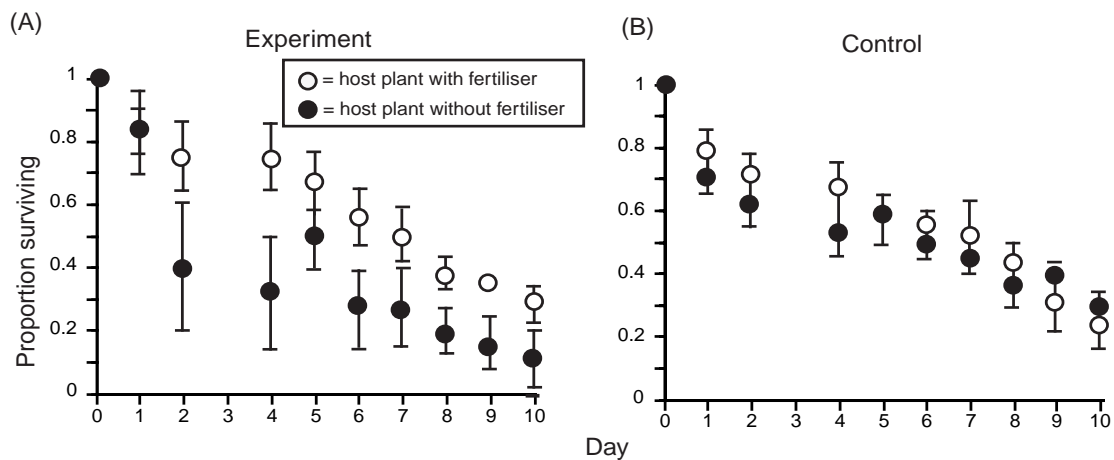
#### Host Plant Quality

Host plant quality can alter the attractiveness of larvae to ants: seedlings of *A. decurrens* were treated with fertilizer, thereby creating foliage that was rich in nitrogen and other co-varying nutrients ('high quality plants') compared with their untreated counterparts ('low quality plants'). Fifth instar larvae on high quality plants attracted  $7.9 \pm 0.8$  ants per larva, whereas those on low quality plants attracted only  $6 \pm 0.3$  ants per larva ( $F_{1,8} = 5.29$ ,  $P = 0.05$ ). Moreover, ant-associated larvae on high quality plants also survived better than their counterparts on low quality plants (Fig. 18.13). Presumably the higher quality host plants sustained caterpillars that produced higher quality and/or more copious secretions than their counterparts feeding on lower quality plants (Baylis and Pierce 1991).

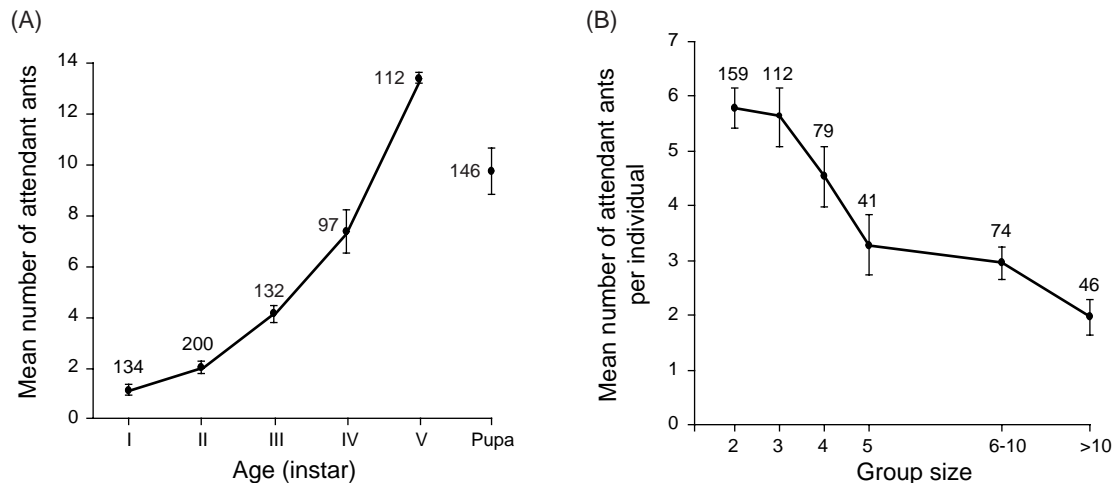
In a similar vein, third and fourth instar larvae of the North American lycaenid, *Glaucopsyche lygdamus* (Doubleday) are tended by a greater number of workers of *Formica altipetens* Francoeur when feeding on seed pods of their host plants, *Lupinus floribundus* Greene, than when feeding on other parts of the same host plants, and it is possible that this difference in ant attendance is a function of the quality of the part of the plant upon which the larvae are feeding (Pierce and Eastal 1986). Moreover, individuals of the same species feeding on different species of host plants can also vary in their attractiveness to ants (Fiedler 1990). This could have important consequences for host selection and/or the ability to switch hosts over evolutionary time (Pierce 1985; Burghardt and Fiedler 1996).

#### Age, Size And Sex

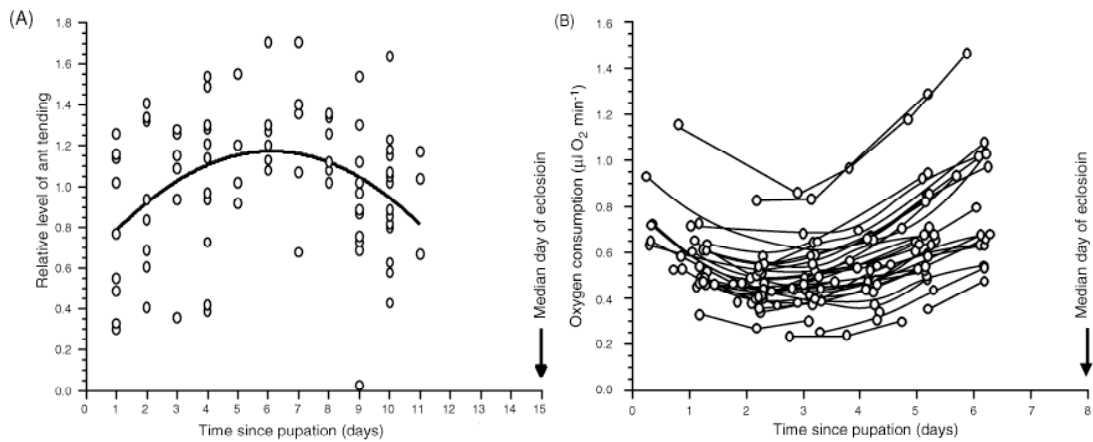
The number of ants associated with a larva increases exponentially with instar (Fig. 18.14A) (Pierce *et al.* 1987). In a more fine-tuned analysis, relative age was also found to affect the attractiveness of pupae to ants. Individuals of *J. evagoras* are less attractive to workers of *I. anceps* shortly after pupation, reach a peak of attractiveness in the middle of the pupation cycle, and then decrease in attractiveness shortly before eclosion (Fig. 18.15A) (Mathews 1993). This relationship is the reverse of that displayed by pupal respiration, in which oxygen consumption is greater shortly after pupation and just before eclosion (Nash 1989) (Fig. 18.15B). Secreting attractants for attendant ants is presumably considerably less costly metabolically than the physiological rearrangements associated with pupation and eclosion. Finally, female pupae are larger than male pupae and thus attract more ants. However, when the effects of size are factored out, there is no significant difference in attractiveness between male and female pupae (Mathews 1993).



**Figure 18.13** Third level trophic interactions between larvae of *J. evagoras*, associated workers of *Iridomyrmex anceps*, lycaenid predators, and host plants of *Acacia irrorata*. Seedlings of *A. irrorata* were treated with fertilizer, thereby creating foliage that was rich in nitrogen and other nutrients ('high quality plants'). Ant attendance and survival of lycaenid larvae on these plants were compared with their counterparts on plants that had not been treated with fertilizer ('low quality plants'). Fifth instar larvae on high quality plants attracted  $7.9 \pm 0.8$  ants per larva, whereas those on low quality plants attracted only  $6 \pm 0.3$  ants per larva ( $F_{1,8} = 5.29$ ,  $P = 0.05$ ). Ant associated larvae on high quality plants also survived better than their counterparts on low quality plants. (A) Points represent means ( $\pm$  SE) of the proportion of larvae surviving on plants with and without fertilizer treatment. (B) In the control treatment, plants were housed in a mesh enclosure, and larvae were reared without ants. The difference in survival of larvae on high and low quality host plants was evident only when larvae were tended by ants and exposed to predation under field conditions. (Redrawn from Baylis and Pierce 1991.)



**Figure 18.14** Mean number of attendant ants ( $\pm$  SE) of *Iridomyrmex anceps* per individual of *J. evagoras* as a function of (A) age and (B) larval aggregation size at a field site in Mt Nebo, Qld. Measurements for juveniles in (A) were taken for solitary individuals, and mean number of attendant ants per individual in (B) were taken for individuals in groups of any age distribution. Samples sizes are provided above. Taken together, the graphs illustrate that a solitary first or second instar larva can gain more ants by joining a group of any size than by remaining on its own. (Redrawn from Pierce *et al.* 1987.)



**Figure 18.15** A comparison of the effect of pupal age on attractiveness to ants versus oxygen consumption. (A) Pupae are more attractive to workers of *I. anceps* (measured as the number of attendant ants in contact with a pupa) in the middle of the pupation period than at either the beginning or the end. (Redrawn from Mathews 1993). Each point represents a pupa marked in the field on the day it pupated and subsequently observed once at a field site in Ebor, NSW. The line shows a second order polynomial regression line fitted to the data ( $r^2 = 0.10$ ,  $F_{2,80} = 5.598$ ,  $P = 0.0053$ ). (B) Pupae consume more oxygen at the beginning and end of the pupal period than in the middle. Lines connect subsequent measurements made on the same pupa ( $n = 39$ ). (Redrawn from Nash 1989.)

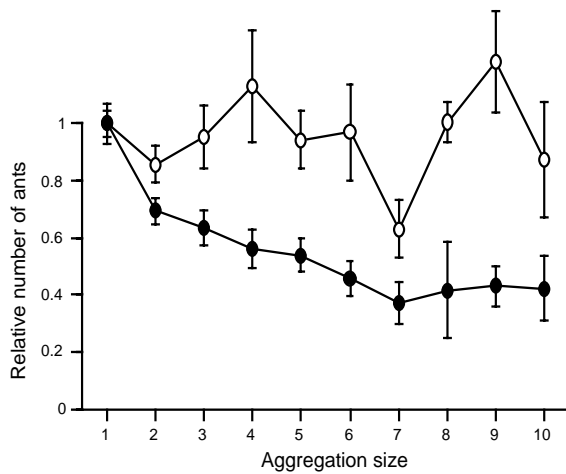
### Social Context

The social context of a larva of *J. evagoras* can also influence the number of attendant ants. Larvae and pupae of *J. evagoras* in larger size aggregations attract fewer attendant ants per individual than their counterparts in smaller aggregations (Fig. 18.14B; Pierce *et al.* 1987). Again, this depends in part upon the behaviour of the ants: attendant ants standing directly 'on' a larva are comparable to the number expected if the larva were found on its own; however, the number of ants standing 'around' the larva, presumably functioning primarily as guards, decreases with increasing group size (Fig. 18.16; Nash 1989). These results are consistent with the observation that larvae strategically control the rate and/or quality of secretion and modify it accordingly, depending upon their social context (e.g. Leimar and Axén 1993; Axén and Pierce 1998).

### Nutritional State of Ant Colony

Lycaenid larvae and pupae that attract attendant ants must compete with alternative food sources in the environment. Thus it is reasonable to hypothesize that the nutritional state of the ant colony will have a strong effect on the likelihood that workers will tend any one of the several trophobionts such as lycaenid larvae and homopterans available in the environment. For example, a colony that has a ready supply of carbohydrates, perhaps from nectaries or extra-floral nectaries of plants, may be less interested in tending carbohydrate-secreting homopterans than a colony without access to plant nectars. The nutritional requirements of a colony may also vary with phenology, depending upon whether workers are rearing other workers, or reproductives in the nest. Ant species may differ in their nutritional requirements depending upon the chemicals they commonly use in communication, or possibly even upon the presence or absence of microbial symbionts. The attractiveness of lycaenid larvae to ants should therefore vary considerably depending upon the nutritional state of the colonies whose workers tend the larvae.

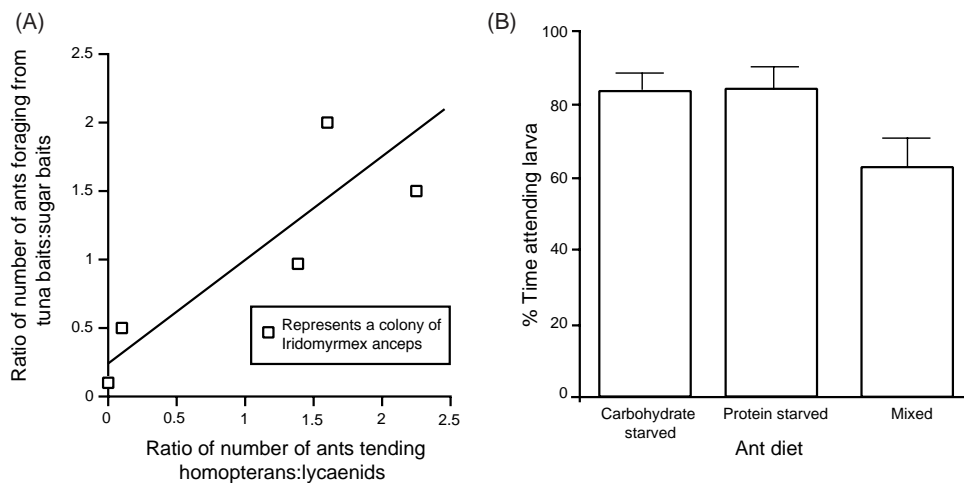
Two kinds of evidence have been gathered to examine the effect of ant nutrition on the attractiveness of larvae of *J. evagoras*. David Nash (1989; Pierce *et al.* 1991) demonstrated under field conditions that colonies of *I. anceps* foraging primarily on carbohydrates from homopterans preferred protein to sugar baits, whereas those foraging primarily on secretions from larvae of *J. evagoras* preferred sugar to protein baits (Fig. 18.17A). In other words, the ant colonies foraged in a way that balanced their overall diet with respect to proteins and carbohydrates. Ann Fraser (1997) pursued this question by



**Figure 18.16** Change in the relative number of ants associated with a group of larvae as a function of aggregation size. Ants are divided into those in physical contact with the larvae ('on'; open circles) and those within 10 mm of but not touching the larval aggregation ('around'; filled circles). The relative number of ants was calculated by dividing the number of ants observed by the number expected if each larva within the aggregation attracted the same ant guard as if it were alone. While the relative numbers of ants standing 'on' the individual larvae were roughly the same whether the individuals were on their own or in aggregations, the relative number of ants 'around' the larvae decreased with increasing group size. (Redrawn from Nash 1989.)

experimentally manipulating the diet of colonies of *I. anceps* under laboratory conditions. She demonstrated that colonies that had been raised for two weeks on either a protein-starved or a carbohydrate-starved diet were more attracted to larvae of *J. evagoras* than those that had been raised on a mixed diet (Fig. 18.17B).

To date, nutritional aspects of attendant ants have only been studied within species, and most of this work has focused on how variation in alternative food resources available to the ants might affect the attractiveness of lycaenid larvae. Additional features such as whether or not colonies are producing



**Figure 18.17** (A) Colonies of *Iridomyrmex anceps* observed in the field appear to balance their diet. The diet of each colony is expressed as the ratio of the number of ants tending lycaenid larvae and pupae to the number collecting honeydew from homopterans. The preference for particular bait types is expressed as the ratio of the number of worker ants that were observed at sucrose baits over a two hour period to the number of workers observed at tuna fish baits during a two hour period. When colonies were foraging primarily on homopteran honeydew, they preferred tuna baits to sugar ones; however, when they were foraging primarily on the secretions of the lycaenid, *J. evagoras*, they preferred sugar baits to tuna ones ( $r^2 = 0.824$ ,  $P < 0.05$ ). (Redrawn from Nash 1989; Pierce *et al.* 1991.) (B) Effect of diet on attendance levels of *I. anceps* ants tending fifth instar larvae of *J. evagoras*. Numbers of attendant ants from 4 colonies of *I. anceps* were measured before and after colonies were reared for 14 days on either tuna (carbohydrate starved), sucrose solution (protein starved) or both (mixed). Responses to diet treatments were not significantly different between colonies, but ant diet had a significant effect on the amount of time ants spent tending larvae. Ants from colonies reared on a mixed diet spent less time tending than those reared on either a protein starved or a carbohydrate starved diet ( $F_{2,81} = 3.55$ ,  $P = 0.03$ ). (Redrawn from Fraser 1997.)

sexuials might also influence their motivation to tend lycaenid larvae. More significantly, different species of ants vary considerably in their dietary requirements, and this has undoubtedly played a crucial role in the evolution of more specialised interactions (Pierce 1989).

### Colony Size

The relationship between colony size and number of ants tending larvae of *J. evagoras* has not been systematically explored under field conditions or using queenright colonies. However, Merrill (1997) demonstrated that nests of *I. anceps* of different sizes exhibited correspondingly different tending levels. The number of workers in contact with a caterpillar increased with nest size, with a mean ( $\pm$  SE) of  $7.0 \pm 0.5$  ants tending larvae in the nests with 80 workers, and  $4.0 \pm 0.3$  ants tending larvae in nests with 15 workers ( $F_{2, 9} = 25.575$ ,  $P < 0.01$ ). Size also affected the percentage of workers in the nest in contact with the caterpillar, with the overall percentage of ants in small nests being almost three times greater than that in large nests ( $F_{2, 9} = 93.209$ ,  $P < 0.001$ ).

Colony abundance and size can influence the relative benefit of one species of attendant ant over another, and may be important determinants of the evolution of highly specialised interactions. Ant species that are more abundant and/or have larger colony sizes represent larger biogeographic 'islands' that can be colonized by myrmecophilous lycaenids. Again, few studies have systematically assessed variation in ecological characteristics of potential ant associates. However, comparisons of numbers of ants tending larvae of *J. evagoras* at six geographic sites occupied by *I. anceps* and six occupied by *I. rufoniger* revealed a suggestive pattern. Tending levels were strikingly different among sites ( $F_{1, 49} = 2.38$ ,  $P = 0.022$ ), but did not differ between ant species ( $F_{1, 49} = 0.07$ ,  $P = 0.79$ ). For example, the overall mean ( $\pm$  SE) of workers of *I. anceps* tending a fifth instar for all six sites was  $6.59 \pm 0.47$ , whereas the overall mean for a fifth instar tended by *I. rufoniger* was  $6.49 \pm 0.36$  (D. Wagner and N.E. Pierce, unpublished results). However, the mean number ( $\pm$  SE) of workers of *I. anceps* tending a fifth instar at the most attractive site was  $8.1 \pm 0.3$ , and at the least attractive site was  $4.5 \pm 0.2$ . Similarly, for *I. rufoniger*, the most attractive site had tending levels of  $7.5 \pm 0.2$ , whereas attendance at the least attractive site was  $4.5 \pm 0.3$ . In other words, the site to site difference in tending levels reflects a complex mosaic of high and low quality patches for colonizing butterflies.

## ■ BEHAVIOUR OF JUVENILES

### Aggregation

Like many other lycaenid butterflies with obligate, often species-specific, associations with attendant ants, the larvae of *J. evagoras* are gregarious. Females lay eggs in clusters on host plants, and both larvae and pupae form aggregations. Aggregation behaviour is affected by both ant attendance and larval age. As described earlier, young larvae (first, second and third instars) aggregated significantly more often during the ant exclusion experiments on plants with ants than on plants without ants, whereas the behaviour of the older instars (fourth and fifth instars) was unaffected by ant presence/absence (Pierce *et al.* 1987; Baylis 1989; Nash 1989).

Fifth instars that are about to pupate, however, are also influenced by the presence or absence of ants, and this behaviour shows a high degree of fine tuning depending upon the density of ants. Carper (1989; Pierce *et al.* 1991) provided pupating larvae with the choice of pupating on their own versus pupating beside another individual which had pupated one day previously. At the same time, she manipulated the ant density experienced by the pupating larvae by altering the ant flow rates to their host plants. On plants where the ant flow rate was relatively high (about five ants per minute), individual larvae chose to pupate alone. On plants where the flow rate was low (about two ants per minute), they chose to pupate beside another individual. On plants where ants were excluded entirely, pupating larvae also were more likely to aggregate. Interestingly, for single larvae allowed to pupate on host plants with no other individuals present, there was a significant positive correlation between the size of the pupating larva and the height on the tree at which it pupated: larger individuals pupated higher on the tree, regardless of ant density (Carper 1989; Pierce *et al.* 1987; Pierce *et al.* 1991). In

summary, the behaviours of pupating larvae clearly seem to reflect strategic decision-making based on their assessment of ant densities.

The mechanism by which larvae and pupae aggregate was further explored by Mathews (1993), who tested whether larvae followed ant pheromone trails, perhaps as a means of finding conspecifics. In this experiment, attendant ants were induced to lay a pheromone trail on one arm of a Y-shaped branch. A third instar larva was released below the Y-junction, and observed while it ascended the branch. The results showed that third instar larvae can detect and follow ant trails: 40 out of 60 chose the arm that had previously contained pupae and had been trail-marked by the attendant ants (Mathews 1993). If ant trails are an important means by which larvae of *J. evagoras* find each other, it is possible that juveniles of *J. evagoras* are less likely to aggregate when ants are experimentally excluded because once the ant foraging trails are extinguished, the larvae have difficulty locating conspecifics. The difference in behaviour of the older instars, however, suggests that they could be using alternative cues such as stridulation or larval silk trails to find each other. It is also possible that the comparative benefits of aggregation are diminished once a certain critical size or age is reached.

### Stridulation

Even in the absence of ant trails, larvae of *J. evagoras* may have means of detecting the locations of conspecific juveniles with which to aggregate. As described before, both the late instar larvae and the pupae of *J. evagoras* are known to stridulate. Travassos (1997) investigated the role of vibratory communication in *J. evagoras*. He 'silenced' pupae by painting shellac over the stridulatory organs of a group of pupae, and compared the attractiveness of these pupae to a group that was allowed to stridulate normally. Pupae that had been experimentally silenced were discovered more slowly by attendant ants, and were significantly less attractive than their singing counterparts.

The larvae have proved to be more difficult to study experimentally since their stridulatory organs appear to be located on almost every segment. However, the significance of their three calls types might be explored in a context-dependent manner by the use of appropriate playback experiments.

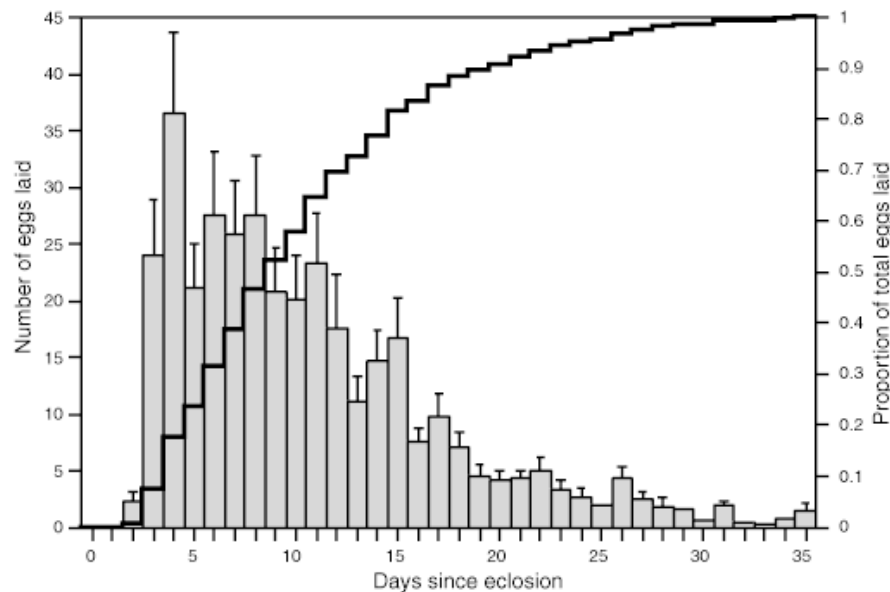
## ■ ADULT BEHAVIOURAL ECOLOGY

### Adult Nutrition and Fecundity

When a female of *J. evagoras* emerges from the pupal stage, she already has a full complement of mature (chorionated) eggs in her ovaries (Hill and Pierce 1989). This is not always the case in the Lepidoptera, and may reflect both the rich diet consumed by the larval stages, and selection for females to lay eggs as rapidly as possible after emergence and mating. The number of mature eggs at eclosion is correlated with female size, as is the number of eggs laid over a female's lifetime, no matter what her adult diet (Hill and Pierce 1989).

The availability of sugar in the adult food of *J. evagoras* can greatly increase the longevity and fecundity of females of *J. evagoras*. In an experiment to examine the importance of adult nutrition in the ecology of *J. evagoras*, females were brought into the laboratory and fed varying concentrations of carbohydrates (fructose, glucose and sucrose in a 2:2:1 ratio) and amino acids (alanine, arginine, glycine, lysine, proline and serine) (Hill and Pierce 1989). Sugar had a major effect, increasing fecundity as much as threefold (one extremely large female laying a record total of 950 eggs during her lifetime), and longevity as much as sevenfold, from four to 28 days. However, amino acids had no effect on either of these variables, although their presence in the diet did stimulate females to feed more often than on water controls. The presence of sugars in their food also stimulated females to feed, and they appeared to compensate for low sugar diets by feeding for longer periods. Individuals lived longer on diets containing a medium level of sugar (25% by weight), but had reduced longevity on higher sugar concentrations. Finally, egg weight was not affected by either sugars or amino acids in the diet, but was correlated with the initial weight of the female, and diminished during her lifetime (Hill and Pierce 1989).





**Figure 18.18** The relationship between the age of females of *J. evagoras* and oviposition. Bars represent the mean daily egg production ( $\pm$  SE) by females of a particular age. The thick line represents cumulative egg production. (After Hill and Pierce 1989.)

These results indicate that adult nutrition can have a significant effect on the population dynamics of *J. evagoras*. In their study of a marked population of individuals, Elgar and Pierce (1988) estimated that females lived for only 3–4 days in the field, which corresponds roughly to the longevity measured by Hill and Pierce (1989) for females with only water available to them in the laboratory. Based on laboratory measurements, a female will lay only 15–20% of her lifetime total number of eggs in four days. That the maximum potential rate of egg-laying does not tail off rapidly over the life of laboratory females demonstrates the desirability, from a female's viewpoint, of maximising her longevity by exploiting nectar sources. If a female can live for only half her laboratory lifetime (about 12 days), she may be able to lay 70% of her lifetime potential number of eggs (Fig. 18.18) (Hill and Pierce 1989).

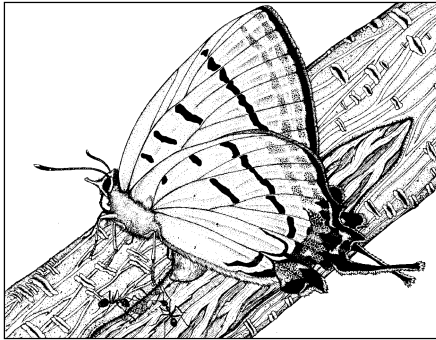
### Oviposition

The great majority of egg masses laid by females of *J. evagoras* are lodged in holes and crevices in the bark of the *Acacia* host plants, and most of them near the base of the primary trunk of the plant, although egg masses are also deposited on leaves and in shallow cavities produced at the point where the leaf attaches to the stem. *Jalmenus evagoras* overwinters in the egg stage. From January through March, 1984, at a study site in Mt Nebo, Queensland, Elgar and Pierce found that females changed their preferences for particular oviposition sites during the course of the season. In the middle of the summer, 86% of the egg masses were laid on the outside of the plants, either on the surface of the leaves or the trunk. This figure fell to 49% by the end of the season, when a greater proportion of eggs were laid in secure cracks and holes in the trunk at the base of the host plant ( $\chi^2 = 9.45$ ,  $df = 3$ ,  $P < 0.03$ ).

Females of *J. evagoras* appear to use a set of hierarchical cues in deciding where to lay eggs: the appropriate host plant species, the nutritional quality of the individual plant, the presence or absence of attendant ants, and the presence or absence of conspecific juveniles all influence her decision.

### Attendant Ants

Considering the importance of attendant ants to the survival of juveniles of *J. evagoras*, it is not surprising that females of *J. evagoras* are influenced by ants in their choice of where to lay eggs. In experiments at Mt Nebo, Queensland, females were offered a choice between 10 host plants of *A. irrorata*. Ants were allowed to forage on conspecific larvae on five of these plants, and were



		Trees with ants	Trees without ants
I.	Eggs laid ( <i>N</i> )	618	104
	Masses	30	4
	Eggs/mass	20.6 ± 4.1	26.0 ± 8.4
II.	Eggs laid ( <i>N</i> )	548	160
	Masses	28	5
	Eggs/mass	19.6 ± 4.4	32.0 ± 4.8

**Figure 18.19** Oviposition by females of *J. evagoras* in response to trees containing conspecifics that are not tended versus trees where conspecifics are tended by workers of *Iridomyrmex anceps*. Results are shown for two experiments, I and II. Each experiment lasted eight days, with treatments swapped after four days (see text). Females were far more likely to lay egg masses on plants containing workers of *I. anceps*; however, the number of eggs per egg mass did not differ between plants with and without ants. (From Pierce and Elgar 1985; illustration by C. W. Adams.)

experimentally excluded on the other five. The density of conspecific larvae was held constant at six fifth instar per plant. After four days, the treatment for each plant were swapped, so that each experiment ran for a total of eight days. Females laid many more egg masses on ant-infested plants, although the clutch sizes of individual egg masses did not vary between treatments (Fig. 18.19) (Pierce and Elgar 1985).

Females responded to the presence or absence of ants before they alighted on prospective plants; once they landed, the probability of oviposition was the same whether or not ants were present. When juvenile homopterans of the membracid, *Sextius virescens* Fairmaire, were used to attract workers of *I. anceps* onto host plants, females preferred to lay eggs on plants with homopterans and ants than on plants with homopterans alone (Pierce and Elgar 1985). Ant-dependent oviposition has been documented in a number of other lycaenid species (Atsatt 1981a; Henning 1983; Pierce and Elgar 1985; Wagner and Kurina 1997); but it does not appear to extend to all myrmecophilous species (Pierce and Eastal 1986).

Not only can females of *J. evagoras* respond to the presence or absence of ants on their host plants, but they can also detect the difference between different species of ants, as well as different populations of the same species. For example, Fraser (1997) offered females of *J. evagoras* raised with workers of *I. anceps* from Nathan, Queensland, a choice between four treatments: host plants containing conspecifics tended by foragers of *I. anceps* from Nathan, *I. anceps* from Canberra, *I. rufoniger* from Ebor, or no ants at all. In two separate trials, females from Nathan preferred to lay eggs on trees occupied by their natal ant associates, *I. anceps* from Nathan (Trial 1:  $G = 34.05$ ,  $df = 3$ ,  $P < 0.001$ ; Trial 2:  $G = 25.57$ ,  $df = 3$ ,  $P < 0.001$ ).

#### Nutritional Quality of the Host Plant

Females are also sensitive to the nutritional quality of the host plant when laying eggs. This was determined in the experiment described earlier where two sets of potted host plants were raised, one treated with fertiliser, and the other not. The leaves of plants treated with fertiliser had a higher nitrogen and water content than the leaves of the unfertilised plants (and were likely to have varied in their concentrations of other compounds not measured in the study). Females laid more egg masses on plants treated with fertiliser than on their poorer quality counterparts, although the number of eggs per egg mass did not vary between the two treatments (Baylis and Pierce 1991). A possible long-range cue that females use before alighting upon a plant is leaf colour. The intensity of green colour was ranked on a subjective scale of 1 to 7 by an independent observer (without knowledge of whether or not they had been treated with fertiliser), and the leaves of the plants in the fertilised treatment were deemed to be a deeper green than those in the unfertilised treatment (Baylis and Pierce 1991). Once a female has alighted upon a plant, presumably short-range cues gained from taste receptors in her tarsi and in the sensory organs around her ovipositor enable her to assess host plant quality.

### Conspecifics

The juvenile stages of *J. evagoras* aggregate, and several studies have investigated whether the presence or absence of conspecifics has an influence on female oviposition behaviour. In a field trial at Mt Nebo, Queensland, M.A. Elgar and N.E. Pierce (unpublished results) investigated whether the presence or absence of conspecific eggs could influence female oviposition. Twelve potted plants of *A. irrorata* that had been matched for age and size were arranged in a circular arena about 3 m in diameter. Previously, six of the plants had been 'pre-treated' by fastening pupae of *J. evagoras* to their branches and allowing workers of *I. anceps* to attend them. Females had laid eggs on these plants, and thus each plant contained  $8.67 \pm 3.2$  egg masses before the start of the experiment, and the positions and numbers of eggs in these 'older' egg masses were noted. Six fifth instars were then placed on each plant, and these were actively tended by workers of *I. anceps*. After seven days, the number of new eggs and egg masses were found not to differ between the two treatments (trees with eggs:  $4.0 \pm 1.1$  egg masses; trees without eggs:  $3.5 \pm 1.3$  egg masses; Mann-Whitney U-Test,  $Z = 0.48$ , ns). Thus females do not appear to respond to the presence of conspecific eggs on the host plant.

The presence of larvae and/or pupae, however, does appear to influence a female's decision to lay eggs, although in the case of at least one ant associate, this is primarily a secondary effect that is mediated through the influence of conspecifics on ant densities on a host plant, rather than a direct effect of the conspecifics themselves. The effects of ant attendance and pupal density were tested in a blocked, two factor experiment conducted at two field sites near Ebor, NSW where larvae are tended by *I. anceps* (Mathews 1993). Females were offered a choice of six potted plants of *A. melanoxyton*, three with ants and three without ants, and each trio consisting of one plant with no pupae, one with one pupa, and one with four pupae. Five replicates of this design were placed at each site. Only ant density significantly explained variance in oviposition behaviour. Because ant densities are also highly correlated with pupal densities, females appear to have a 'dosage-dependent' response to ant numbers (Mathews 1993).

### Mating

As described earlier, the mating system of *J. evagoras* has been shaped in significant ways by the close association between juveniles of the species and attendant ants. The protection afforded by ant guards means that juveniles of *J. evagoras* are unusually predictable and 'apparent' in their locations. For example, larvae feed in aggregations, and pupate in highly visible locations on their host plant. This means that males of *J. evagoras* can readily locate the position of conspecific pupae. Males routinely fly from host to host, apparently 'trap-lining' the location of prospective mates.

To the best of our knowledge, females of *J. evagoras* mate only once. Nevertheless, males routinely inspect and attempt to mate with older females that reject them by turning towards them and vigorously fluttering their wings. Given the frequency with which this behaviour is observed, it is reasonable to suspect that harassing males are sometimes successful in achieving second matings, or in finding unmated females in this manner. However, older females, as estimated from wing wear, have been observed mating on only a very few instances over all the years that the species has been studied in the field, and none of the females that has been dissected has ever contained more than one spermatophore (Hill and Pierce 1989). It seems likely that not all females are mated upon eclosion, and thus selection favors males that constantly patrol the mating status of adult females. Individual males can mate more than once, and factors affecting male mating success have already been discussed. Time of eclosion, relative size and longevity are all critical components of male lifetime reproductive success in this species (Elgar and Pierce 1988).

Shortly before the emergence of the adult, a pupa of *J. evagoras* secretes volatile chemicals that attract males. This can be demonstrated dramatically by crushing a pupa that is about to eclose: males are attracted to alight upon fingers coated with compounds from the crushed pupa. But perhaps the most curious aspect of this attraction is that males do not appear to be able to distinguish between male and female pupae, and will hover about a male pupa until the adult emerges and then discover their mistake through trial and error. Presumably any male that could distinguish between the sexes would be at such an advantage that the ability would rapidly spread through the population. Similarly, any

female that could signal her sexual identity might have a mating advantage, and again, selection would favor the evolution of recognition signals.

Several explanations could account for why lack of sexual discrimination persists. One possibility is that male pupae mimic female pupae in order to distract rival males and thereby increase their own chances of encountering unmated females. However, in a marked population of individuals observed for their entire estimated lifespans, males were never observed to mate on the day that they eclosed themselves; earliest matings began on the day following eclosion (Elgar and Pierce 1988). Thus it is unlikely that by distracting rivals while in the pupal stage, a male could increase his own chances of finding unmated females, since he would not normally have access to those females on the day that he himself eclosed.

Another possible explanation addresses the interests of signaling females. Although males can mate multiple times in *J. evagoras*, second-time mating males remain *in copula* for a significantly longer period than first-time mating males. Average copulation time for males mating more than once increased from 2.39 hours at the first mating ( $N = 78$ ), to 3.05 hours at the second mating ( $N = 44$ ), to 4.01 hours at the third mating (repeated measures ANOVA for  $n = 17$  three-time mated males,  $F_{2,32} = 11.434$ ,  $P < 0.001$ ) (L. Hughes, D. Wagner, B. Chang, and N. Pierce, unpublished results). This presumably represents a considerable cost to a mating female because copulating pairs whose flight is burdened and awkward are particularly vulnerable to predation. The longer a female is *in copula*, the longer she is exposed to this heightened level of vulnerability. Moreover, on the day following a successful mating, the wet weight of the spermatophore of a second-time mating male was on average 26.6% smaller than at the first mating, and represented only 1.7% of the male's eclosion weight (paired *t*-test,  $t = 6.558$ ,  $P < 0.0001$ ,  $df = 21$ ) (L. Hughes, D. Wagner, B. Chang, and N. Pierce, unpublished results). This reduction in spermatophore size did not result in a detectable reduction in egg production, as has been observed in some other species of Lepidoptera.

These two observations, longer copulation duration and lower spermatophore weight, suggest that it may sometimes be disadvantageous for a female to mate with a second-time mating male. If male and female pupae are indistinguishable, competition for mates will be reduced, because, from the perspective of searching males, the potential mating pool is effectively twice as large. Thus larger, longer-lived males would be less able to monopolize all of the matings in a population, and females would be more likely to be mated by first-time mating males. On first glance, this would appear to contradict a 'good genes' argument in which selection should favour females that mate with larger, longer-lived males that have greater success in mating contests. However, if on average the costs of mating with a second-time mating male actually outweigh the potential benefits accrued by his relative size and longevity, selection would not favor efficient recognition.

The importance of attendant ants in shaping the mating system is reflected by the observation that males of *J. evagoras* are influenced by ants when searching for mates. Males were observed approaching and inspecting *A. irrorata* host plants, each containing four fifth instar larvae, in which half of the plants had foraging workers of *I. anceps*, and half had ants excluded. Males preferentially approached and inspected plants containing conspecifics and ants: approximately 65% of all inspections were of plants containing foraging ants ( $N = 150$ ,  $\chi_1^2 = 13.50$ ,  $P < 0.01$ ) (Elgar and Pierce 1988). Adults of both sexes of *J. evagoras* can thus detect and respond to their customary attendant ant associate. The fact that mating occurs on the host plant, and that males use attendant ants as cues in finding mates may have played a role in shaping population structure and divergence of populations over evolutionary time.

## ■ PHYLOGENY AND POPULATION STRUCTURE

### Phylogeny

Attempts to estimate the phylogeny of *Jalmenus* using molecular characters have met with varying degrees of success. An initial effort to estimate relationships was based on characters from the non-coding mitochondrial 'control region' (CR), sometimes called the 'D-loop' region for mammals, or the

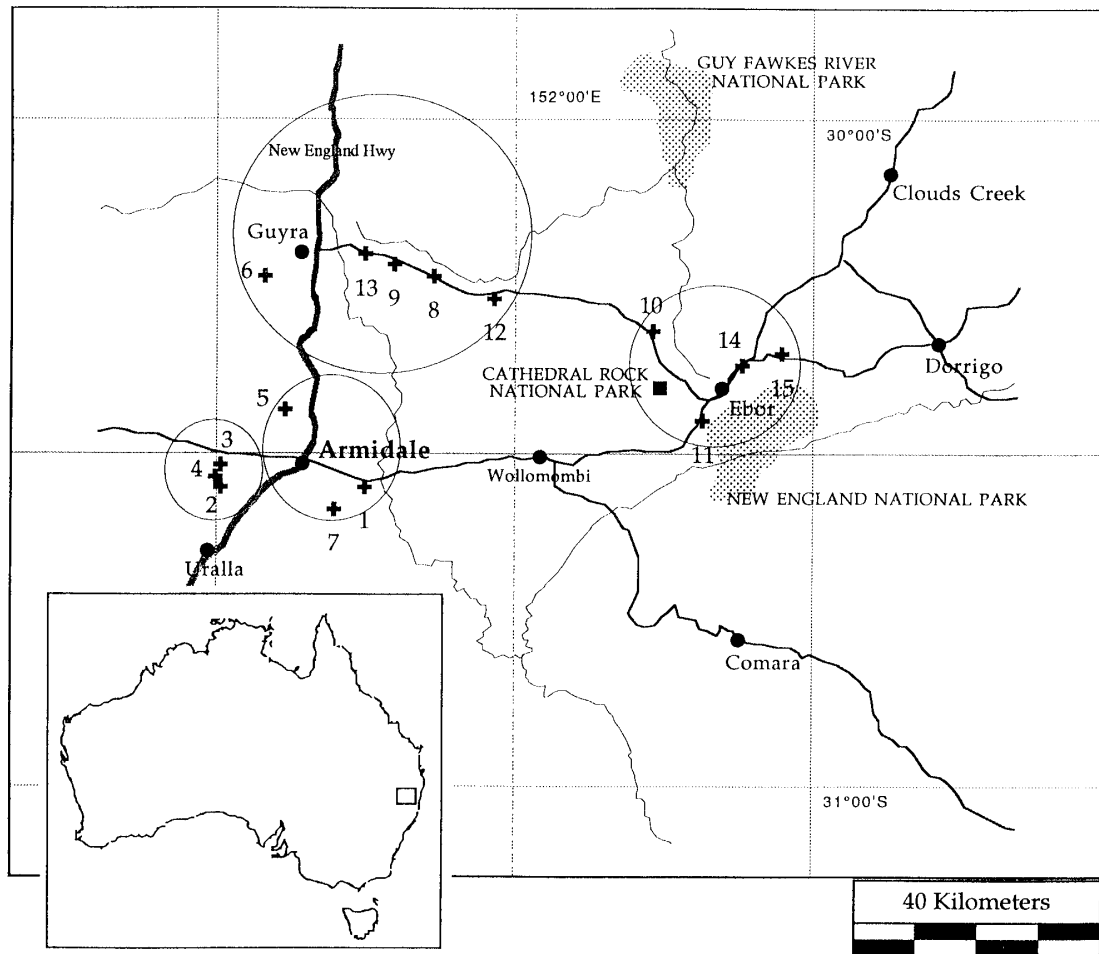
'A+T-rich region' for invertebrates. Preliminary data from nuclear genes suggested that species of *Jalmenus* might be very recently diverged (Braverman 1989), and thus the CR was chosen because it appears to be highly variable in insects, providing a potentially promising source of markers for phylogenetic reconstruction of closely related taxa. In particular, the extensive intraspecific polymorphism of CR length observed in *D. melanogaster* (Hale and Singh 1986), as well as the considerable interspecific divergence in CRs between different species of *Drosophila* (Clary and Wolstenholme 1987) suggested that the CR might be valuable for phylogenetic reconstruction of species of *Jalmenus*. Taylor *et al.* (1993) sequenced about 350 base pairs of CR from several populations each of six species and one subspecies of *Jalmenus*, including *J. pseudictinus* Kerr and Macqueen, *J. daemeli*, *J. ictinus*, *J. evagoras eubulus* Miskin, *J. evagoras evagoras*, *J. icilius* Hewitson and *J. lithochroa* Waterhouse. Analysis of the approximately 250 bp of comparable sequence revealed only 15 variable sites, or essentially 94% sequence identity among the species of *Jalmenus*. Of the 15 variable sites, many were also polymorphic within species. While these data were consistent with a model of rapid diversification put forward on the basis of the ecology and behaviour of the butterflies (Pierce 1984), they were not informative phylogenetically.

A subsequent study using both mitochondrial *Cytochrome oxidase* subunits I and II (COI and II), and nuclear *Elongation factor 1-alpha* (EF1- $\alpha$ ) also suggested recent and rapid diversification in the genus (Mignault 1996; Mignault and Pierce, unpublished results). These genes exhibited remarkably little differentiation among species. Divergences for EF1- $\alpha$  ranged from 0.25% to 2.79%, and variable sites were relatively uninformative phylogenetically, although they recovered *J. aridus* as the sister species to its congeners. Similarly, divergences for pairwise comparisons among species of *Jalmenus* ranged from 0.21% to 2.01% for COI and II. However, most of the variable sites were informative, and thus characters from these genes generated a moderately resolved phylogeny estimate (refer back to Figure 18.1) Data from COI and II also placed *J. aridus* as the sister taxon to the rest of the group. With the exception of *J. aridus*, the taxa from Western Australia were extremely closely related; differences between these species could not be resolved. The three species of *Jalmenus* known to associate with ants in the *I. purpureus* species group and their close relatives formed a clade, raising the possibility that initial association with *I. purpureus* may have in some way constrained the subsequent evolution of this clade. *Jalmenus daemeli* and *J. pseudictinus* were each other's closest relatives, and in addition to feeding on species of *Acacia*, both of these species utilise members of the Sapindaceae as host plants. They associate, however, with different species of ants (*I. rufoniger* group and *F. kirbyi* respectively).

### Population Structure

The influence that ant associations might have on the genetic differentiation of colonies ('sites') and geographically defined 'subpopulations' of *J. evagoras* was explored in an area covering about 150 square kilometers between Armidale and Ebor, NSW (Fig. 18.20) (Costa *et al.* 1996). Colonies of *J. evagoras* commonly associate with two ants in this area, *I. rufoniger* in and around Armidale, and *I. anceps* in and around Ebor. Throughout the region, *J. evagoras* feeds on a number of different species of *Acacia*, predominantly *A. filicifolia*, *A. melanoxydon*, and *A. decurrens*. Any one colony of *J. evagoras* is typically found with only one of the two ant species, and these local associations persist from year to year.

Under laboratory conditions, individuals of *J. evagoras* derived from colonies associating with the two ant mutualists, *I. anceps* and *I. rufoniger*, will mate readily and produce viable offspring (N.E. Pierce, unpublished results). Thus they belong to the same species, and are not, in fact, cryptic species, a condition that would provide alternative explanations for differences between them. This question is important because, although otherwise morphologically indistinguishable, adults of *J. evagoras* associating with *I. rufoniger* are significantly smaller on average than adults associating with *I. anceps*: for colonies tended by *I. rufoniger*, male and female forewing lengths averaged  $20.65 \pm 1.51$  mm (mean  $\pm$  SE) and  $21.84 \pm 2.08$  mm, respectively, whereas for colonies tended by *I. anceps*, males and females measured  $21.14 \pm 1.24$  mm and  $22.85 \pm 1.87$  mm (Nested ANOVA,  $F_{1, 495} = 26$ ,  $P < 0.01$ ) (Costa *et al.* 1996). Moreover, the aggregation behaviour of pupating larvae on trees differed dramatically between sites inhabited by *I. anceps* and *I. rufoniger*: clusters of pupae tended by



**Figure 18.20** Distribution of colonies ('sites') of *J. evagoras* located in and around Armidale and Ebor, NSW. Colonies at sites 1–7 are tended by *I. anceps*, whereas those at sites 8–15 are tended by *I. rufoniger*. Four geographically defined 'subpopulations' are circled. Population structure was analysed using starch-gel electrophoresis for six polymorphic loci. The analysis detected modest genetic structure, with ant associate being a poor predictor of patterns of variation. Geographic patterns of variation are complex, with distance not a major determinant. The patterns are consistent with a scenario involving repeated extinction/recolonization of small local colonies. Because of the extreme patchiness of the butterfly's distribution, recolonization is a highly stochastic process in which the source population is not necessarily the nearest one. (Redrawn from Costa *et al.* 1996.)

*I. anceps* averaged about  $12.21 \pm 20.47$  pupae per cluster, whereas those tended by *I. rufoniger* averaged only about  $6.12 \pm 6.72$  pupae per cluster ( $F_{1, 225} = 17.6$ ,  $P < 0.01$ ) (Costa *et al.* 1996).

Both adult size differences and degree of pupal aggregation may reflect genetic divergence between ant mutualist 'races', or may simply be the outcome of plastic responses to environmental variables, including the species of ant associate. Thus the analysis of genetic structure of colonies of *J. evagoras* in this region began with the premise that at least two sets of factors could influence the genetic characteristics of *J. evagoras* populations: (1) the availability and distribution of 'ant+plant' sites, and (2) incipient race formation associated with attendant ants. The first factor could give rise to the considerable physical patchiness observed in the distribution of subpopulations of *J. evagoras*. The second could result in divergence between ant races as each set of subpopulations becomes specialised to its particular ant associate.

The importance of these factors in shaping the population dynamics of *J. evagoras* was evaluated using six polymorphic allozyme markers. Genetic structure was determined hierarchically, using both

geographic distance and ant associate as separate components of the analysis. The analysis detected modest genetic structure, with ant associate being a poor predictor of patterns of variation. Geographic patterns of variation were complex, with distance not a major determinant. All six loci possessed some alleles exhibiting disjunct frequencies, but none had all alleles exhibiting this pattern. This frequency distribution could have arisen given restricted movement between sites in situations where allele frequencies are subject to drift, such that some become disjunct while others remain similar. The patterns are consistent with a scenario involving repeated extinction/recolonization of small local colonies. Because of the extreme patchiness of the butterfly's distribution, recolonization is a highly stochastic process in which the source population is not necessarily the nearest one (Costa *et al.* 1996).

The observations of behavioural and morphological differences between sites where larvae associate with different ant species are therefore likely to reflect a plastic response by *J. evagoras* to its attendant ant species rather than differentiation along ant-associated lines. However, the possibility of ant association promoting high rates of diversification remains: the patchy ecology of the species and the small numbers of individuals forming each subpopulation result in a pattern of extinction/colonization that has left a distinct population genetic footprint. The genetic structure data, together with estimates of Nei's genetic distance for pairwise site and subpopulation comparisons suggest that populations of *J. evagoras* are spatially and temporally dynamic, setting the stage for a pattern of extinction/recolonization not found in continuously distributed species.

#### ■ FUTURE GOALS

Relationships such as the one between *J. evagoras* and its attendant ants continue to raise questions that can be addressed at multiple levels of analysis. In particular, much more needs to be learned about the ecological, biochemical and morphological factors promoting and maintaining species-specific associations. A full analysis of the secretions used in rewarding and ensuring favorable recognition by appropriate ant species would be particularly fruitful. At a similarly mechanistic level, much remains to be learned about the role of inter- and intra-specific acoustic signaling in these insects. Ecological considerations, such as the relative importance of competition among ants for lycaenid larvae of different species, the influence of ant diet on tending behaviours, and the degree of specialization of ant rewards all remain to be fully explored. The physiological control that ants exert on lycaenid larval development is an essentially open question: the fact that association with ants can trigger early metamorphosis, and that ant-associated larvae are able to invest a significantly larger proportion of their resources into egg production as adults suggests that a close examination of these interactions could give us considerable insight into environmental factors regulating lycaenid development.

Finally, the effect of specialised life histories on population structure would undoubtedly provide us with an understanding not only of mechanisms generating the impressive diversity of Lycaenidae we see today, but also of factors to consider in preserving this rich heritage. Many myrmecophilous lycaenids require appropriate species of associated ants as well as particular host plants. Because most lycaenids are small, their dispersal abilities are also more limited, and their generation times shorter. Adults frequently perch on the host plant, where mating also occurs, and both males and females of highly ant-associated species use ants as cues in laying eggs and recognizing mates.

Many species of Lycaenidae have recently gone extinct or are recognised as endangered, and Lycaenidae are frequently used as emblems for conservation biology, including the large blue in the UK, the Karner blue and the Xerces blue in the US, the Brenton blue in South Africa, and Illidge's blue in Australia, to name a just a few well-known examples. As the work here indicates, highly specialised taxa such as these are more sensitive to environmental perturbations because their lives are so complex: when something changes, there's simply more that can go wrong. Moreover, their constellation of life history specializations creates temporal and spatial patchiness in their distributions, placing constraints on effective population size and promoting situations where population structuring and potential subdivision can occur. A better understanding of population structure, genetic variability, and likelihood of extinction will help us to develop management practices that can minimise the impact of habitat destruction on these and other species with similarly

specialised life histories. Lycaenid–ant interactions can therefore provide model systems for understanding both the mechanisms generating the diversity of species we see today, and the processes that may lead to their extinction.

#### ■ ACKNOWLEDGMENTS AND A BRIEF HISTORY OF RESEARCH ON *J. EVAGORAS*

The research presented here is the cumulative work of many people, and since this is a review of *Jalmenus evagoras*, it seems appropriate to provide a brief account of researchers who have studied the species.

A small population of *J. evagoras* first captured Roger Kitching's interest when he noticed a tree with large aggregations of caterpillars in the middle of a city parking lot in Canberra in the early 70s, and he published a paper describing the ultrastructure of the eggs in 1976. He began to analyse the population biology of the species in earnest in 1979, after Tony Hiller had told him about a population of *J. evagoras* on Mt Nebo, Qld. Work on this population has continued for almost 20 years. By coincidence, knowing of her interest in lycaenid–ant interactions, Phil Ward had written a letter to Naomi Pierce in 1977 extolling the virtues of *J. evagoras* and its associated ants as a model system for research. Thus when Pierce visited Kitching and his lab group in 1979 (including Martin Taylor, who completed the first honours thesis which included work on *J. evagoras* in 1980), this system became a focus for her doctoral research, which was completed in 1983. Since that time, 7 doctoral dissertations and 11 honours or senior theses have included studies of *J. evagoras* (Table 18.3).

Others who have published research specifically on *J. evagoras* include Peter Atsatt, Michael Braby, Ralf Buckley, Jim Costa, Phil DeVries, Mark Elgar, Marty Kreitman, Trevor Hawkeswood, John McDonald, John Smiley, Diane Wagner and Bill Young. In addition, those who have worked, or still work on different aspects of the species include Nevil Amos, Tim Anderson, Tom Bellas, Joy Bergelson, Andrew Berry, David Brown, Dana Campbell, Simon Easteal, Simon Emms, Jay Evans, Graham Hopkins, Lesley Hughes, Takao Itino, Matt Kane, Roxanna Keen, David Lohman, Michael Magrath, Paul Mead, Kim Miller, Ben Normark, Karen Nutt, Bert Orr, Peter Rogers, Leslie Schenkel, Man-Wah Tan, Tom Tregenza, Peter Vowles, Diane Wagner, Nina Wedell and David Wilson.

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In the preparation of this manuscript, Roger Kitching and Ed Seling took the SEM photographs shown in Figure 18.2 and 18.4 respectively. Mark Travassos recorded the sonograms shown in Figures 18.3 and 18.4. Andre Mignault prepared the essential elements of Figure 18.1. Christopher Adams made the black and white drawings shown in Figures 18.3, 18.4 and 18.19, and assisted in the creation of all of the figures. Hiltrud Engel prepared the histological sections shown in Figure 18.2. Jenifer Bush edited the text. The ideas were improved by comments from Andrew Berry, Dana Campbell, Brian Farrell, Ann Fraser, Roger Kitching, David Lohman and Diane Wagner. Joy Bergelson, Belinda Chang, Mark Elgar, Ann Fraser, Lesley Hughes, Jeff Mathews, David Merrill, Andre Mignault, Mark



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This paper is dedicated to the memory of our colleague and friend, Robin Carper (1966–1997), who helped us appreciate aspects of the complexity of this system and many others, and at the same time uncovered some elegantly simple truths.

TABLE 18.3. Dissertations involving research on *Jalmenus evagoras*.

Doctoral theses		
1983	Naomi Pierce Harvard University	The ecology and evolution of symbioses between lycaenid butterflies and ants
1987	Chris Hill Griffith University	The effect of adult diet on the biology of butterflies
1989	Matthew Baylis Oxford University	The role of nutrition in an ant–lycaenid–host plant interaction.
	David Nash Oxford University	Cost-benefit analysis of a mutualism between Lycaenid butterflies and ants
1993	Jeff Mathews Oxford University	Aggregation and mutualism in insect herbivores
1997	Ann Fraser Harvard University	Evolution of specialization in lycaenid butterfly–ant mutualisms
1998	AnnKristen Axén University of Stockholm	To be determined
Senior theses and honours projects		
1980	Martin Taylor Griffith University	The foraging behaviour of <i>Iridomyrmex anceps</i> Roger
1984	Kim Benbow Griffith University	Aggregation in a myrmecophilous lycaenid butterfly, <i>Jalmenus evagoras evagoras</i> (Donovan) 1805 (Lepidoptera: Lycaenidae)
1988	Belinda Chang Princeton University	Some determinants of female fecundity in <i>Jalmenus evagoras</i> (Lepidoptera: Lycaenidae)
	Francine Laden Princeton University	Host plant effects: can species differences in host plants influence the attractiveness of lycaenid butterflies to ants?
1989	John Braverman Princeton University	DNA sequence variation and evolutionary radiation in the Australian genus <i>Jalmenus</i> (Lepidoptera: Lycaenidae)
	Elizabeth Carper Princeton University	The effects of varying levels of ant attendance on the aggregation behavior and survivorship of larvae of <i>Jalmenus evagoras</i> (Lepidoptera: Lycaenidae)
1996	André Mignault Harvard University	Proposed genealogical relatedness among butterflies of the Australian genus <i>Jalmenus</i> (Lepidoptera: Lycaenidae) — A case study in phylogenetic inference, ecology and biogeography of a rapidly evolving system
	Daniel Smith Harvard University	The interactive effect of elevated CO <sub>2</sub> and drought on the growth and physiology of three Australian tree species in the genus <i>Acacia</i>
	Trinh Quach Harvard University	An investigation of aggregation patterns of <i>Jalmenus evagoras</i> larvae experiencing varying levels of ant attendance and the role of kin recognition in larval aggregation
1997	David Merrill Harvard University	Deception in lycaenid–ant mutualism?
	Mark Travassos Harvard University	Calling caterpillars, pulsing pupae: vibratory communication in the Common Imperial Blue butterfly, <i>Jalmenus evagoras</i> (Lepidoptera: Lycaenidae)

## ■ REFERENCES

- Andersen, A.N., 1995. A classification of Australian ant communities, based on functional groups which parallel plant life-forms in relation to stress and disturbance. *J. Biogeography* **22**: 15–29.
- Atsatt, P.R., 1981a. Ant-dependent food plant selection by the Mistletoe Butterfly *Ogyris amaryllis* (Lycaenidae). *Oecologia* **48**: 60–63.
- Atsatt, P.R., 1981b. Lycaenid butterflies and ants: selection for enemy-free space. *Amer. Nat.* **118**: 538–654.
- Axén, A.H., Liemar, O. and Hoffman, V., 1996. Signalling in a mutualistic interaction. *Animal Behaviour* **52**: 321–333.
- Axén, A.H., 1998. In preparation. Doctoral dissertation for the University of Stockholm.
- Axén, A. and Pierce, N.E., 1998. Aggregation as a cost reducing strategy for lycaenid larvae. *Behav. Ecol.* **9**: 109–115.
- Ballmer, G.R. and Pratt, G.F., 1991. Quantification of ant attendance (myrmecophily) of lycaenid larvae. *J. Res. Lepid.* **30**: 95–112.
- Barbosa P. and Schultz, J.C. (eds), 1987. *Insect outbreaks*. 578 pp. Academic Press, San Diego.
- Baylis, M., 1989. *The role of nutrition in an ant-lycaenid-host plant interaction*. PhD thesis, Oxford University.
- Baylis, M. and Pierce, N. E., 1991. The effect of host plant quality on the survival of larvae and oviposition behaviour of adults of an ant-tended lycaenid butterfly, *Jalmenus evagoras*. *Ecol. Entomol.* **16**: 1–9.
- Baylis, M. and Pierce, N. E., 1992. Lack of compensation by final instar larvae of the myrmecophilous lycaenid butterfly, *Jalmenus evagoras*, for the loss of nutrients to ants. *Physiol. Entomol.* **17**: 107–114.
- Baylis, M. and Pierce, N.E., 1993. The effects of ant mutualism on the foraging and diet of lycaenid caterpillars. Pp. 404–421. In: Stamp, N.E. and Casey, T.M. (eds) *Caterpillars: Ecological and Evolutionary Constraints on Foraging*. vi + 587 pp. Chapman and Hall, New York.
- Benbow, K.F., 1984. *Aggregation in a myrmecophilous lycaenid butterfly, Jalmenus evagoras evagoras (Donovan) 1805 (Lepidoptera: Lycaenidae)*. Honours dissertation, Griffith University.
- Bhatkar, A. and Whitcomb, W.H., 1971. Artificial diet for rearing various species of ant. *Fla. Entomol.* **53**: 229–232.
- Braby, M.F., 1988. New food plants for *Jalmenus evagoras evagoras* (Donovan) (Lepidoptera: Lycaenidae). *Aust. Entomol. Mag.* **15**: 33–34.
- Braby, M.F., 1989. The butterfly fauna of La Trobe University, Victoria. *Victorian Nat.* **106**: 118–132.
- Braby, M.F., 1998. Notes on the biology of some Hesperidae and Lycaenidae (Lepidoptera) in South-eastern Australia. *Victorian Nat.* **115**: 4–8.
- Brain, P. and Maslin, B.R., 1996. A serological investigation of the classification of *Acacia* subgenus Phyllodinae (Leguminosae: Mimosoideae). *Biochem. Syst. Ecol.* **24**: 379–392.
- Braverman, J.M.N., 1989. *DNA sequence variation and evolutionary radiation in the Australian genus Jalmenus (Lepidoptera: Lycaenidae)*. Senior thesis, Princeton University.
- Bremer, K., 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**: 795–803.
- Burghardt, F. and Fiedler, K., 1996. The influence of diet on growth and secretion behaviour of myrmecophilous *Polyommatus icarus* caterpillars (Lepidoptera: Lycaenidae). *Ecol. Entomol.* **21**: 1–8
- Carper, E.R., 1989. *The effects of varying levels of ant attendance on the aggregation behavior and survivorship of larvae of Jalmenus evagoras (Lepidoptera: Lycaenidae)*. Senior thesis, Princeton University.
- Chang, B.S-W., 1988. *Some determinants of female fecundity in Jalmenus evagoras (Lepidoptera: Lycaenidae)*. Senior thesis, Princeton University.
- Clary, D.O. and Wolstenholme, D.R., 1987. Drosophila mitochondrial-DNA-conserved sequences in the AT-rich region and supporting evidence for a secondary structure model of the small ribosomal-RNA. *J. Mol. Evol.* **25**: 116–125.
- Common, I.F.B. and Waterhouse, D.F., 1981. *Butterflies of Australia*, 2nd ed., xiv + 682 pp. Angus and Robertson, Sydney.
- Costa, J.T., McDonald, J.H., and Pierce, N.E., 1996. The effect of ant association on the population genetics of the Australian butterfly *Jalmenus evagoras* (Lepidoptera: Lycaenidae). *Biol. J. Linn. Soc.* **58**: 287–306.
- Cottrell, C.B., 1984. Aphytophagy in butterflies: its relationship to myrmecophily. *Zool. J. Linn. Soc.* **79**: 1–57.
- Crosby, D.F., 1994. New distribution and food plant records for some Victorian butterflies (Lepidoptera: Hesperioidea, Papilionoidea). *Aust. Entomol.* **21**: 65–68.
- Cushman, J.H., Rashbrook, V.K. and Beattie, A.J., 1994. Demonstration of benefits to both participants in a lycaenid-ant association. *Ecology* **75**: 1031–1041.
- DeVries, P.J., 1990. Enhancement of symbioses between butterfly caterpillars and ants by vibrational communication. *Science* **248**: 1104–1106.
- DeVries, P.J., 1991. Mutualism between *Thisbe irenea* butterflies and ants, and the role of ant ecology in the evolution of larval-ant associations. *Biol. J. Linn. Soc.* **43**: 178–195.
- DeVries, P.J. and Baker, I., 1989. Butterfly exploitation of an ant-plant mutualism: adding insult to herbivory. *Journal of the New York Entomol. Society* **97**, 332–340.
- Donoghue, M.J., Olmstead, R.G., Smith, J.F., and Palmer, J.D., 1992. Phylogenetic relationships of Dipsacales based on rbcL sequences. *Annals of the Missouri Botanical Garden* **79**: 333–345.
- Downey, J.C., 1966. Sound production in pupae of Lycaenidae. *J. Lepid. Soc.* **20**: 129–155.

- Downey, J.C. and Allyn, A.C. 1973. Butterfly ultrastructure: 1. Sound production and associated abdominal structures in pupae of Lycaenidae and Riodinidae. *Bulletin of the Allyn Museum* **14**: 1–47.
- Downey, J.C. and Allyn, A.C., 1978. Sound produced in pupae of Lycaenidae. *Bulletin of the Allyn Museum* **48**: 1–13.
- Dunn, K.L., 1984. *Acacia diffusa* Lindl. — A new larval foodplant for *Jalmenus evagoras evagoras* (Donovan) (Lepidoptera: Lycaenidae). *Victorian Entomol.* **14**: 8.
- Dunn, K.L. and Dunn, L.E., 1991. *Review of Australian Butterflies: distribution, life history and taxonomy. Part 3 Lycaenidae.* iii+336–512 pp. Privately published.
- Eastwood, R. and Fraser, A.M., *in review*. Ant-butterfly associations in Australia. *Aust. J. Ecol.*
- Elgar, M.A. and Pierce, N.E., 1988. Mating success and fecundity in an ant-tended lycaenid butterfly. Pp. 59–75. *In*: Clutton-Brock, T.H. (ed.) *Reproductive success: Studies of selection and adaptation in contrasting breeding systems.* ix + 538 pp. University of Chicago Press, Chicago.
- Evesham, E.J.M., 1985. The interaction of food distribution and the caste composition of an ant colony (*Myrmica rubra* L.). *J. Zool., London (A)* **207**: 241–250.
- Fiedler, K., 1988. The preimaginal epidermal organs of *Lycaena tityrus* (Poda, 1761) and *Polyommatus coridon* (Poda 1761) (Lepidoptera: Lycaenidae) — a comparison. *Nota Lepidopterol.* **11**: 100–116.
- Fiedler, K., 1990. Effects of larval diet on myrmecophilous qualities of *Polyommatus icarus* caterpillars (Lepidoptera: Lycaenidae). *Oecologia* **83**: 284–287.
- Fiedler, K., 1991. *Systematic, evolutionary and ecological implications of myrmecophily within the Lycaenidae* (Insecta: Lepidoptera: Papilionoidea) Bonner Zoologisches Monographien 31.
- Fiedler, K. and Maschwitz, U., 1988. Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and lycaenids (Lepidoptera: Lycaenidae). II. Lycaenid larvae as trophobiotic partners of ants — a quantitative approach. *Oecologia* **75**, 204–20.
- Fiedler, K. and Maschwitz, U., 1989. Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and lycaenids (Lepidoptera: Lycaenidae) I. Release of food recruitment in ants by lycaenid larvae and pupae. *Ethology* **80**: 71–80.
- Fiedler, K., Seufert, P., Pierce, N.E., Pearson, J.G. and Baumgartner, H-T. 1995. Exploitation of lycaenid-ant mutualisms by braconid parasitoids. *J. Res. Lepid.* **31**: 153–168.
- Fraser, A.M., 1997. Evolution of specialization in lycaenid butterfly-ant mutualisms. PhD thesis, Harvard University.
- Graham, A.J. and Moulds, M.S., 1988. A new species of *Jalmenus* Hübner (Lepidoptera: Lycaenidae) from Western Australia. *Gen. Appl. Entomol.* **20**: 57–62.
- Greenslade, P.J.M., 1979. *A guide to the ants of South Australia.* x + 44 pp. South Australian Museum, Special Education Bulletin Series, Adelaide.
- Greenslade, P.J.M., 1985. Some effects of season and geographical aspects on ants (Hymenoptera: Formicidae) in the Mt. Lofty ranges, South Australia. *Trans. R. Soc. Aust.* **109**: 17–23.
- Hale, L.R. and Singh, R.S., 1986. Extensive variation and heteroplasmy in size of mitochondrial-DNA among geographic populations of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* **22**: 8813–8817.
- Hawkeswood, T.J., 1981. The food plants of *Jalmenus evagoras* (Donovan) (Lepidoptera: Lycaenidae). *Aust. Entomol. Mag.* **8**: 1–2.
- Henning, S.F., 1983. Chemical communication between lycaenid larvae (Lepidoptera: Lycaenidae) and ants (Hymenoptera: Formicidae). *J. Entomol. Soc. South. Afr.* **46**: 341–366.
- Henning, S.F., 1987. Myrmecophily in lycaenid butterflies (Lepidoptera: Lycaenidae). *Entomol. Rec. and J. of Variation* **99**: 215–222
- Hill, C.J., 1987. The effect of adult diet on the biology of butterflies. PhD thesis, Griffith University.
- Hill, C.J., 1993. The myrmecophilous organs of *Arhopala madytus* Fruhstorfer (Lepidoptera, Lycaenidae). *J. Australian Entomol. Soc.* **32**: 283–288.
- Hill, C.J. and Pierce, N.E., 1989. The effect of adult diet on the biology of butterflies, 1: The common imperial blue, *Jalmenus evagoras*. *Oecologia* **81**: 249–257.
- Johnson, S.J., Hay, R.W. and Bollam, H.H., 1992. *Jalmenus notocrucifer* sp.n. (Lepidoptera: Lycaenidae) from south Western Australia. *Aust. Entomol. Mag.* **19**: 69–74.
- Jordano, D., Rodriguez, J., Thomas, C.D., and Haeger, J.F., 1992. The distribution and density of a lycaenid butterfly in relation to *Lasius* ants. *Oecologia* **91**: 439–446.
- Kitching, R.L., 1976. The ultrastructure of the eggs of *Jalmenus evagoras* (Donovan) (Lepidoptera: Lycaenidae). *Aust. Entomol. Mag.* **3**: 42–44.
- Kitching, R.L., 1983. Myrmecophilous organs of the larvae of the lycaenid butterfly *Jalmenus evagoras* (Donovan). *J. Nat. Hist.* **17**: 471–481.
- Laden, F., 1988. *Host plant effects: Can species differences in host plants influence the attractiveness of lycaenid butterflies to ants?* Senior thesis, Princeton University.
- Leimar, O. and Axén, A. H., 1993. Strategic behaviour in an interspecific mutualism: interactions between lycaenid larvae and ants. *Anim. Behav.* **46**: 1177–1182.
- MacKay, W.P., 1985. A comparison of the energy budgets of three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). *Oecologia* **66**: 484–494.
- Malicky, H., 1969. Versuch einer analyse der ökologischen beziehungen zwischen Lycaeniden (Lepidoptera) und Formiciden (Hymenoptera). *Tijdschr. Entomol.* **112**: 213–298.

- Malicky, H., 1970. New aspects of the association between lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymenoptera). *J. Lepid. Soc.* **24**: 190–202.
- Maschwitz, U., Wust, M., and Schurian, K., 1975. Blaulingsraupen als Zuckerlieferanten für Ameisen. *Oecologia* **18**, 17–21.
- Mathews, J.N.A., 1993. *Aggregation and mutualism in insect herbivores*. DPhil thesis, Oxford University.
- Merrill, D.N., 1997. *Deception in lycaenid-ant mutualism?* Senior thesis, Harvard University.
- Mignault, A.A., 1996. *Proposed genealogical relatedness among butterflies of the Australian genus Jalmenus (Lepidoptera: Lycaenidae) — A case study in phylogenetic inference, ecology and biogeography of a rapidly evolving system*. Senior thesis, Harvard University.
- Nash, D.R., 1989. *Cost-benefit analysis of a mutualism between lycaenid butterflies and ants*. DPhil thesis, Oxford University.
- Nijhout, H.F., 1975. A threshold size for metamorphosis in the tobacco hornworm, *Manduca sexta* (L.). *Biol. Bull.* **149**: 214–225.
- Nijhout, H.F., 1981. Physiological control of molting in insects. *Amer. Zool.* **21**: 631–640.
- Pierce, N.E., 1983. *The ecology and evolution of symbioses between lycaenid butterflies and ants*. PhD thesis, Harvard University.
- Pierce, N.E., 1984. Amplified species diversity: a case study of an Australian lycaenid butterfly and its attendant ants. Pp. 197–200. In: Vane-Wright, R.I and Ackery P. (eds.) *The biology of butterflies*. xxv + 429 pp. Academic Press, London.
- Pierce, N.E., 1985. Lycaenid butterflies and ants: selection for nitrogen-fixing and other protein rich food plants. *Am. Nat.* **125**: 888–895.
- Pierce, N.E., 1989. Butterfly–ant mutualisms. Pp. 299–324. In: Grubb P.J. and Whittaker, J. (eds.) *Towards a more exact ecology*. x + 468 pp. Blackwell, Oxford.
- Pierce, N.E., 1995. Predatory and parasitic Lepidoptera: carnivores living on plants. *J. Lepid. Soc.* **49**: 412–453.
- Pierce, N.E. and Easteal, S., 1986. The selective advantage of attendant ants for the larvae of a lycaenid butterfly, *Glaucopsyche lygdamus*. *J. Anim. Ecol.* **55**: 451–462.
- Pierce, N.E. and Elgar, M.A., 1985. The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid butterfly. *Behav. Ecol. Sociobiol.* **16**: 209–222.
- Pierce, N.E., Kitching, R.L., Buckley, R.C., Taylor, M.F., and Benbow, K., 1987. Costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras* and its attendant ants. *Behav. Ecol. Sociobiol.* **21**: 237–248.
- Pierce, N.E., Nash, D.R., Baylis, M., and Carper, E.R., 1991. Variation in the attractiveness of lycaenid butterfly larvae to ants. Pp. 131–143. In: Cutler, D. and Huxley, C. (eds) *Ant-plant interactions*. xviii + 601 pp. Oxford University Press, Oxford.
- Playford, J., Appels, R., and Baum, B.R. 1992. The 5S DNA units of *Acacia* species (Mimosaceae). *Plant Syst. Evol.* **183**: 235–247.
- Quach, T. M., 1996. *An investigation of aggregation patterns of Jalmenus evagoras larvae experiencing varying levels of ant attendance and the role of kin recognition in larval aggregation*. Senior thesis, Harvard University.
- Shattuck, S.O., 1992a. Generic revision of the ant subfamily Dolichoderinae (Hymenoptera: Formicidae). *Sociobiology* **21**: 1–181.
- Shattuck, S.O., 1992b. Review of the dolichoderine ant genus *Iridomyrmex* Mayr with descriptions of three new genera (Hymenoptera: Formicidae). *J. Aust. Entomol. Soc.* **31**: 13–18.
- Shattuck, S.O., 1993. Revision of the *Iridomyrmex purpureus* species-group (Hymenoptera: Formicidae). *Invertebr. Taxon.* **7**: 113–149.
- Slatkin, M., 1977. Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology* **12**: 253–263.
- Smiley, J.T., Atsatt, P.R., and Pierce, N.E., 1988. Local distribution of the lycaenid butterfly, *Jalmenus evagoras*, in response to host ants and plants. *Oecologia* **76**: 416–448.
- Smith, D., 1996. *The interactive effect of elevated CO<sub>2</sub> and drought on the growth and physiology of three Australian tree species in the genus Acacia*. Senior thesis, Harvard University.
- Swofford, D. L., 1993. *PAUP, Phylogenetic Analysis Using Parsimony*, Version 3.1, Laboratory of Molecular Systematics, Smithsonian Institution.
- Tauber, C. A. and Tauber, M. J., 1989. Sympatric speciation in insects: Perception and perspective. Pp. 307–344. In: Otte D. and Endler J.A. (eds) *Speciation and its consequences*. xiii + 679 pp. Sinauer Associates, Sunderland.
- Taylor, M.F.J., 1980. The foraging behaviour of *Iridomyrmex anceps* Roger. Honours dissertation, Griffith University.
- Taylor, M.F.J., McKechnie, S.W., Pierce, N.E., and Kreitman, M.E., 1993. The lepidopteran mitochondrial control region: Structure and evolution. *Mol. Biol. Evol.* **10**: 1259–1272.
- Taylor, R.W., 1987. *A checklist of the ants of Australia, New Caledonia and New Zealand (Hymenoptera: Formicidae)*. 92 pp. CSIRO Australia Division of Entomology Report No. 41.
- Thomas, J.A., Elmes, G.W., Wardlaw, J.C., and Woyciechowski, M., 1989. Host specificity among *Maculinea* butterflies and *Myrmica* ant nests. *Oecologia* **79**: 452–457.
- Tindale, M.D. and Roux, D.G., 1974. An extended phytochemical survey of Australian species of *Acacia*: chemotaxonomic and phylogenetic aspects. *Phytochemistry* **13**: 829–239.
- Travassos, M., 1997. *Calling caterpillars, pulsing pupae: Vibratory communication in the Common Imperial Blue Butterfly, Jalmenus evagoras (Lepidoptera: Lycaenidae)*. Senior thesis, Harvard University.

- Wagner, D., 1994. *Species-specific effects of tending ants on the life history of Hemiargus isola, a North American lycaenid butterfly*. PhD thesis, Princeton University.
- Wagner, D., and Kurina, L., 1997. The influence of ants and water availability on oviposition behaviour and survivorship of a facultatively ant-tended herbivore. *Ecological Entomology* **22**: 352–360.
- Yamaguchi, S., 1988. *The life histories of five myrmecophilous lycaenid butterflies of Japan*. 262 pp. Kodansha, Tokyo.
- Zar, J.H., 1996. *Biostatistical analysis —Third edition*. x+662 pp. Prentice Hall, Upper Saddle River, New Jersey.