antidepressant drugs (*15*). Our results suggest that some of these clinical failures may be the result of an alteration in ovarian hormone release or response. Support for this is provided by the recent report indicating that estrogen administration is effective in treating depressed women who have failed to respond to more conventional therapies (*16*). Further studies will be necessary to determine whether this interaction is peculiar for imipramine or also influences the action of other antidepressant drugs.

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Parasitoids as Selective Agents in the Symbiosis Between Lycaenid Butterfly Larvae and Ants

Abstract. *The larvae of* Glaucopsyche lygdamus *(Lepidoptera: Lycaenidae) secrete substances that attract ants. In two field sites in Colorado, tending ants protect caterpillars of* G. lygdamus *from attack by braconid and tachinid parasitoids. This protection may have been an important feature in the evolution of the association between lycaenid larvae and ants.*

The caterpillars of many species in the butterfly family Lycaenidae secrete substances that attract ants. Previous accounts of interactions between these taxa include descriptions of the associations and of the histology of the glands (*1-3*). There have been few experimental inquiries into the behavioral and ecological mechanisms underlying the evolution of this symbiosis (*4, 5*).

We present evidence that tending ants protect the caterpillars of *Glaucopsyche lygdamus* Doubleday from attack by parasitoid insects. This protection may act as a potent selective force in maintaining the symbiosis between these lycaenid larvae and ants.

Typically, a cryptic, grublike larva feeding on flowers is surrounded by a retinue of ants that groom it and palpate it with their antennae (Fig. 1). The seventh abdominal segment of the larva bears a dorsal gland, Newcomer's organ, that oozes a honeydew which the ants harvest (*6, 7*). Recent study suggests that regions of the caterpillar eliciting palpation are covered with epidermal glands which secrete substances that attract and appease ants (*6*). Occasionally, the larva everts a pair of hairlike tentacles from the eighth segment, and these might act as defensive structures when the honeydew gland has been depleted or if the larva is alarmed (*6, 8*).

Parasitoids such as tachinid flies and braconid wasps can attack a caterpillar of *G. lygdamus* during any stage of its development. Once infected, a larva survives until just before or immediately after pupation, at which point the parasitoids emerge and kill their host.

Controversy exists concerning the nature of the advantage of being tended by ants. Some argue that tending ants might ward off potential parasitoids (*1, 2*), while others assert that the larvae produce appeasement substances and honeydew simply to escape from ant predation (*6*). Our observations indicate that, by producing attractive substances, *G. lygdamus* caterpillars secure ant defense against parasitoid attack.

Experiments were performed in two habitats in Gunnison County, Colorado, where *G. lygdamus* utilizes different larval food plants and interacts with different species of ants. The "Gold Basin" site, at 2300 m, about 16 km southwest of Gunnison, is a dry region where the chief woody vegetation is sagebrush with scattered aspen groves. Here, *G. lygdamus* feeds primarily on the developing inflorescences of *Lupinus floribundus* Greene, and is tended primarily by the ant *Formica altipetens* Wheeler. The "Naked Hills" site, at 2900 m in Gothic, is a wet alpine meadow where *G. lygdamus* feeds on the flowers, seed pods, and leaves of *Lupinus bakeri* Greene, and is tended largely by workers of *Formica fusca* Linnaeus (*9*).

In both places, ants began tending the larvae at the third instar, and almost all larvae were tended. At this stage, we excluded ants from an experimental group of caterpillars by coating the lupine stems with a viscous barricade of bird or tree Tanglefoot (Tanglefoot Co., Grand Rapids, Michigan) to prevent ants from ascending to the upper leaves, seed pods, and flowers where the caterpillars were feeding. A halo, 0.5 m in radius, was clipped around the base of each plant to eliminate grass bridges that might provide access to the larvae. Controls were treated in the same manner, except that the Tanglefoot was applied on only one side of the stem so that ants could still reach the larvae. Only infested lupines were used in each area, and plants were designated alternatively control or experimental. A total of 106 lupines at Gold Basin and 46 lupines at Naked Hills were monitored during the experiment. Larval densities were not manipulated. Many larger plants with multiple inflorescences contained more than one larva, but seldom more than three or four (*10*).

Experimental and control caterpillars were censused every third day until they reached the final instar, whereupon they were collected. Only one larva was collected from any single inflorescence. The

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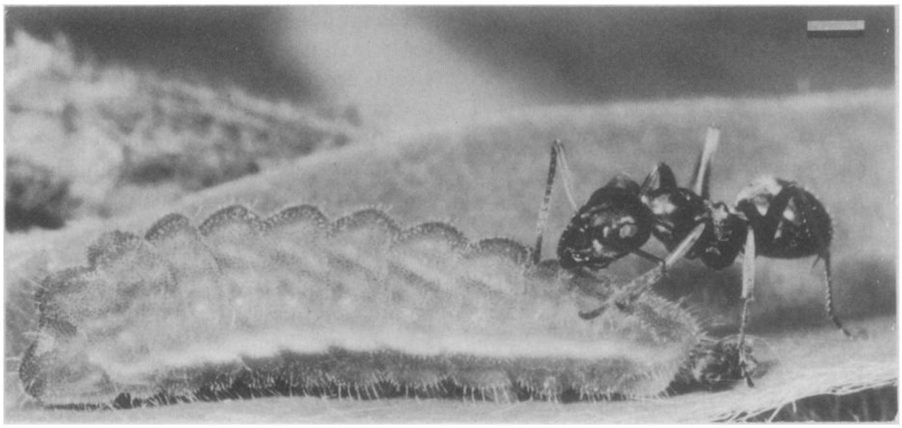

Fig. 1. *Formica fusca* tending a final instar larva of *Glaucopsyche lygdamus*. The ant is drinking from the honeydew gland. The bar indicates 1 mm.

experimental period lasted 6 days in most instances, although it ranged from 3 to 12 days, depending on the developmental rates of the caterpillars. Because of their high disappearance rate, consistently fewer experimental caterpillars, as compared to controls, were retrieved, but the ratio was similar for each census. After collection, larvae were placed in individual petri dishes in a growth chamber and, within a few days, either pupated or died when parasitoids emerged.

A significantly higher proportion of untended (as compared to tended) larvae was attacked by parasitoids (Table 1). Ants were also observed defending caterpillars (Fig. 2). The parasitoids were either braconid wasps, *Apanteles cyaniridis* Riley, or tachinid flies, *Aplomya theclarum* Scudder. Both parasites attack other lycaenid species and are not restricted to *G. lygdamus* (*11*). Ant protection was especially effective against braconids, which are smaller than the ants. In Gold Basin, braconids alone were able to attack 37 percent ($N = 38$) of the experimental caterpillars, but only 7 percent ($N = 57$) of those tended by ants.

Table 1. Results of experiments comparing the parasitism rates of ant tended versus untended larvae of *Glaucopsyche lygdamus*. Ants were excluded by means of a viscous barricade applied to the base of the plants of the experimental group. At Gold Basin, *G. lygdamus* is tended by the ant *Formica altipetens*, and at Naked Hills, by *F. fusca*. In both experiments, the parasitism rate of the untended group was significantly higher. The first number in the parentheses indicates the percentage of parasitism by braconid wasps, and the second number indicates the percentage of parasitism by tachinid flies.

Site	Larvae without ants		Larvae with ants	
	Parasitized (%)	Total (N)	Parasitized (%)	Total (N)
Gold Basin	42* (37, 5)	38	18 (7, 11)	57
Naked Hills	48† (11, 37)	27	23 (0, 23)	39

*$\chi^2_1 = 6.92$; $P < .01$. †$\chi^2_1 = 4.51$; $P < .04$.

In addition, many caterpillars on experimental plants disappeared completely between censuses, while few disappeared from control plants. Of 217 initial experimental larvae monitored at Gold Basin, only 38 survived until the final instar and were collected. Of the initial 156 controls monitored, 57 were retrieved ($\chi^2_1 = 17.31$; $P < .005$). Whether this difference is due to ant protection from predators such as sphecid wasps that have been observed to prey on caterpillars in both sites, or from other causes such as larvae dropping off the plants when they are not tended by ants, remains to be determined.

The pattern of protection observed in the experimentally manipulated environment was also reflected in a natural setting. A random sampling of final instar larvae and their attendant ants was made at Naked Hills over a period of 1 week. The parasitism rate of untended caterpillars or those tended by only one ant was 63 percent ($N = 27$), whereas the rate of those tended by three or more ants was only 26 percent ($N = 38$) ($\chi^2_1 = 5.60$; $P < .02$). This measurement does not give information about the past history of ant attendance. However, individually marked ants in the field were observed to be remarkably constant in their attention to a given larva, and once a tending relationship had been established, subsequent parasitism of the larva did not appear to disrupt the association.

Ants are known to guard plants secreting extrafloral nectar (*12*). Intricate obligate mutualisms have evolved in which ants protect plants from herbivores and competitors, and in turn are fed and housed by the plants (*13*). Similarly, the honeydew gland of the lycaenids can be viewed as a kind of nectary, providing rewards to ants in return for protection from parasitoid attack. Our experiments show that tending ants can have a substantial impact on the survivorship of lycaenid caterpillars, although the importance of this in the overall population dynamics of the butterfly remains to be seen.

Before calling their association mutualistic, the nutritive value to the ants of the lycaenid honeydew must be quantified. The ants may benefit from the honeydew which is produced at a cost to the larvae. Alternatively, it is possible that the larvae are receiving the services of the ant guard for very little in exchange; rather than providing an important food resource to the ants as do plant nectaries and their homopteran analogs aphids, coccids, and membracids, the larvae may simply be mimicking these more abundant and valuable resources (*14*). By providing only a minimal sugar bait, the comparatively rare and widely dispersed caterpillars could take advantage of the characteristic fidelity with which ants defend important food resources (*15*). The fact that tending and guarding nectaries already form an integral part of the behavioral repertory of many ant species might have facilitated the evolution of opportunistic bribery and perhaps even outright deception on the part of some Lycaenidae. Epidermal glands producing attractive substances alone might be adequate to attract ant protection. In this regard, several lycae-

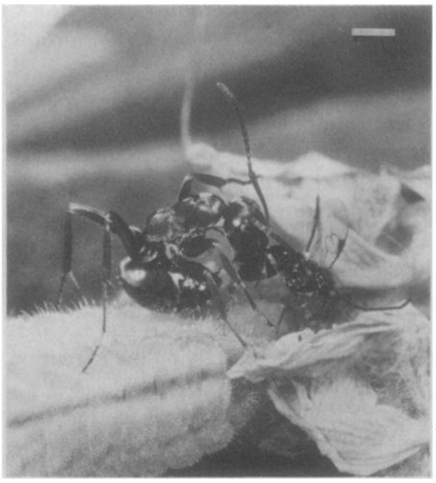

Fig. 2. *Formica fusca* defending a final instar larva of *Glaucopsyche lygdamus*. The ant has seized an attacking braconid wasp parasitoid in its mandibles, and is doubled over in its effort to squirt the wasp with defensive compounds from the tip of the gaster. The bar indicates 1 mm.

nid species that are tended by ants but do not possess honeydew glands have been described (*15*). Like myrmecophilous staphylinid beetles (*16*) and other ant associates, certain lycaenid species such as *G. lygdamus* might simply be exploiting the ants which tend them.

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10. A χ^2 goodness of fit revealed no significant difference in parasitism rates of caterpillars retrieved from individual inflorescences of the same plant versus those retrieved from inflorescences of separate plants.
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Dye Transfer Through Gap Junctions Between Neuroendocrine Cells of Rat Hypothalamus

Abstract. *Most magnocellular neurosecretory cells that terminate in the posterior pituitary secrete either vasopressin, oxytocin, or enkephalin. Intracellular injection of the fluorescent dye Lucifer Yellow into single magnocellular neurons in slices of rat hypothalamus resulted in dye transfer between these cells. Freeze-fracture replicas of these cells occasionally revealed gap junctions, which presumably contain channels that mediate the dye coupling. These two independent techniques strongly suggest that some mammalian neuropeptidergic cells are electrotonically coupled, providing a possible means for recruitment and synchronization of their electrical activity.*

Magnocellular neurosecretory cells (MNC's) in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the mammalian hypothalamus synthesize the neuropeptides oxytocin, vasopressin, and enkephalin (*1, 2*) as three immunohistochemically distinct populations (*3*). In the unstimulated rat, MNC's fire action potentials irregularly at a low rate. Increased discharge in characteristic spike patterns is associated with neurohormone release from terminals in the neurohypophysis (*4, 5*). Specifically, a pulse of oxytocin, which is released by a synchronous burst (30 to 80 Hz lasting 1 to 4 seconds) of action potentials, causes milk ejection in the lactating female (*4*). Under conditions requiring water conservation, vasopressin is thought to be released through a gradual recruitment of MNC's into a "phasic" bursting pattern (10 to 20 Hz lasting 30 to 60 seconds) (*5*). Several mechanisms underlying these bursting patterns can be suggested. Local circuit neurons or fibers from extrahypothalamic inputs might synaptically drive the neurosecretory cells. Alternatively, nonsynaptic mechanisms, such as endogenous oscillations in membrane conductance (*6*) or fluctuations in the concentration of extracellular ions (*7*), could cause changes in membrane potential and associated burst discharge. Finally, electrotonic coupling could mediate synchrony (*8, 9*) and contribute significantly to burst activity within a population of cells (*8*).

The morphological substrate of electrotonic coupling between cells in a variety of tissues is the gap junction, which contains channels that permit dyes and other molecules of < 1000 daltons to pass between cells without entering extracellular space (*10*). Indirect physiological evidence for electrotonic coupling and ultrastructural observations of gap junctions in thin section have been reported for neurons in several areas of the mammalian brain (*11*). It is not known whether mammalian neuroendocrine cells (*12*) or any other hypothalamic cells possess electrotonic junctions. In the study reported here we used single-cell injections of the fluorescent dye Lucifer Yellow CH (457 daltons) (*13, 14*) and the freeze-fracture technique and found that some MNC's are dye-coupled and possess gap junctions. This provides two independent lines of evidence for electrotonic coupling between these mammalian neuropeptidergic cells.

Since first histologically defined (*15*), the hypothalamo-neurohypophysial system has served as the primary model for examination of brain peptide synthesis, storage, and release (*2*). However, our understanding of the electrophysiology of this system has been limited by the difficulties associated with obtaining intracellular recordings in the hypothalamus of intact mammals. Recently it was shown that the brain slice is a suitable preparation for impaling neurons in the PVN of the rat hypothalamus (*16*). The elimination of vascular pulsation and stereotaxic problems permits the intracellular recording and dye injection essential for the study of cell-to-cell coupling.

Magnocellular neurosecretory cells of the PVN (*17*) or SON were first injected with Lucifer Yellow and then identified by their minimum diameter of 15 μm and their large cytoplasm-to-nucleus ratio (Fig. 1). Of 32 well-filled MNC's, 14 were dye-coupled to a second MNC. The proportions of dye-coupled cells in the nuclei were similar (9 of 19 injected cells in the PVN, 5 of 13 in the SON). Of these, 4 of 9 injected cells were dye-coupled in males and 10 of 23 in females. The site of dye coupling appeared to be either dendrodendritic or dendrosomatic (Fig. 1A), although three pairs of MNC's had close cell body apposition (Fig. 1, B and C) suggesting somasomatic coupling

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