SYMBIOTIC RELATIONSHIP BETWEEN Anthene emolus (LYCAENIDAE) AND Oecophylla smaragdina (FORMICIDAE): AN OBLIGATE MUTUALISM IN THE MALAYSIAN RAINFOREST

By

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This document is dedicated to Justin Saarinen and the Heffernan family.
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The larvae of Anthene emolus (Lycaenidae) cannot survive in the wild without their associated ant Oecophylla smaragdina (Formicidae). The ants groom the lycaenid larvae for secretions and transport young larvae around the host plant. Both major and minor ants tend larvae, although the frequencies of tending by different castes proved to be significantly different at different instars. Ants of the same colony were observed fighting among themselves when it came to imbibing lycaenid larval secretions. Major ants showed aggression against the minor ants that were trying to tend the secreting larvae. Major ants precluded minors from the most nutritious secretions, leaving minors to tend the less productive, early instars. On a few occasions, major ants killed larvae they were tending (presumably accidentally).

When larvae were experimentally reared with only one caste of ant, the resulting butterflies showed no differences in dry weight. Mortality was high in larvae reared with
only minor ants. Larvae that were experimentally reared without ants took longer to pupate.

The presence of aggression between intra-colonial caste members poses interesting questions about the allocation of tasks in weaver ants. It also interjects the question why both castes of ants instinctively tend larvae. Future research with tropical ants is necessary to elucidate the specifics of such elaborate, obligate symbioses.
CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

General Information on Lycaenidae

The Lycaenidae (including Riodinidae) is an exceptional family of butterflies containing 40% of all known butterfly species (Vane-Wright, 1978). This is one of the most speciose of all the Papilionoidea families, with an estimated 6000 species (Shields, 1989). About 75% of the species with a known life history associate with ants (Pierce et al., 2002). These ant associations and specialized behavior may have contributed to the high rates of speciation in this family (Fiedler, 1991; Pierce et al., 2002).

Lycaenidae are worldwide throughout most tropical and temperate regions. Some genera of these butterflies are confined to the Oriental Region, although other genera may have circumtropical or particular Old World area distribution (Corbet and Pendlebury, 1992). This thesis focuses on a species from a pan-Southern Hemisphere genus that occurs in the Malay Peninsula, a particularly speciose area for the Lycaenidae (Corbet and Pendlebury, 1992; Fleming 1975).

Lycaenid butterflies are relatively small and are commonly referred to as blues, coppers, hairstreaks, and harvesters. The upper-side wing coloration of males is typically blue, but may range to deeper purple, green, orange, brown, or white. Females are typically more drably colored, but shades of dull blue are still commonly found on the upper wing surfaces. Sexual dimorphism almost always occurs as a difference in pattern on the upper wing surface (Corbet and Pendlebury, 1992). In addition, both sexes of
many species have filamentous tails, which may be used as a camouflage protection to confuse predators (Fleming, 1975).

Lycaenid larvae are commonly referred to as being “wood louse” shaped, or onisciform. There is considerable diversity in the form of these larvae, but most have an elongated prothorax and retractable head (Ballmer and Pratt, 1988). Larvae may possess some of a variety of specialized organs that attract or appease ants (Pierce et al., 2002). These are termed myrmecophilous organs, and are discussed later.

**General Information On Formicidae**

Ants are members of the family Formicidae (Hymenoptera). They are eusocial insects; they demonstrate a reproductive division of labor, cooperative brood care, and overlap of generations (Bourke, 1988; Hölldobler and Wilson, 1990). Ants are some of the most dominant members of a region’s fauna. They are also arguably one of the most highly social of all organisms.

There are close to 9,000 described species of ants worldwide. A large number of species remain undescribed, especially in the tropics. New species and even new genera of ants are still being discovered and described (Hölldobler and Wilson, 1990). There are approximately 120 genera of ants in Malaysia (Dr. Seiki Yamane, personal communication). Ants account for a large number of species, and also for a great biomass. Ant colonies exist in a wide variety of habitats, and colonies may be upwards of one million in number. Ants, other Hymenoptera, and termites account for over 75% of the total insect biomass (Hölldobler and Wilson, 1990). This thesis focuses on a particular species of ant, *Oecophylla smaragdina*, which is discussed later.
**Hypotheses on Lycaenid-Ant Interactions**

Several hypotheses have been proposed to explain the relationship between lycaenids and ants. Malicky (1970) supported the “defense hypothesis,” whereby lycaenids are myrmecophilous so they may survive in the presence of aggressive ants. He did not believe that the attendant ants afforded any protection to the larvae, but rather tolerated them.

Others propose that the relationship is mutually beneficial to both the ants and the lycaenid larvae, and this has been shown in several cases (Pierce and Mead, 1981; Pierce and Eastseal, 1986; Pierce et al., 1987). These studies show how the larvae benefit from the protection of an ant guard that deters parasitoids and predators. Ants benefit from the nutritious secretions of the larvae.

**Important Terms**

The relationship that exists between lycaenid larvae and ants has been referred to as a symbiosis (Ballmer and Pratt, 1991). A symbiosis is “the intimate, relatively protracted, dependent relationship of members of one species with those of another” (Hölldobler and Wilson, 1990, p.643). Symbioses may be further divided into commensalism, mutualism, and parasitism (Hölldobler and Wilson, 1990). The wide variety of ant and lycaenid relationships includes examples in all these categories. However, the overall term used to define ant-lycaenid relationships is myrmecophily, meaning “ant loving.” This is a bit of a misnomer, as lycaenid larvae may actually be parasitic on ants. Additionally, ants may have a positive effect on the lycaenids, and the descriptive term “lepidopterophily” may be more fitting (DeVries, 1987).

The terminology of symbioses and myrmecophily has often come into question and there have been several attempts to clarify and universalize the language (Fiedler, 1991;
Pierce et al., 2002). Myrmecophily may be divided into obligate and facultative forms. Obligate myrmecophiles are members of species that are dependent on ants under natural field conditions, and require association with ants during some part of their lives. Obligate associations often involve only one species or genus of ant. Ants involved in these associations are generally longer-lived, dominant species, which form large colonies (Fiedler, 1991). Additionally, mutualistic and parasitic relationships are likely to be considered obligate (Pierce et al., 2002).

Facultative myrmecophily is a looser relationship. Facultative ant associates do not require the presence of ants for their survival, and associated ants may be from different species or genera. Most ant-lycaenid larvae interactions appear to be facultative (Pierce et al., 2002).

Another term used in ant-lycaenid relationships is myrmecoxeny. This term is used to describe larvae that possess an ant-associated organ, but that are not tended by or observed to associate with ants. An ant-organ may be present to prevent ant aggression, but no tending is observed in myrmecoxenous lycaenids (Kitching and Luke, 1985; Pierce et al., 2002).

As mentioned earlier, lycaenids may be parasitic on their ant partners. Cottrell (1984) described a variety of ways larvae may act aphytophagously. Larvae may wholly or partly refrain from eating the young shoots or flowers that are the norm, and instead eat insect-derived food sources. These may include ant brood, ant regurgitations, Homoptera and/or their honeydew; or they may be cannibalistic on each other. Pierce (1995) also described carnivory in larval lycaenids as a result of the close ant association.
Larvae may feed on ant brood, a high-quality food source, which enables them to produce nutritious secretions for the adult ants to imbibe.

**Specialized Myrmecophilous Organs**

Specialized organs mediate the relationships between lycaenid larvae and ants. Larvae may exhibit all, none, or a variation of such organs. All lycaenid larvae possess an extended prothorax and retractable head. They all also have a thickened cuticle relative to other Lepidopterous larvae. This is presumed to protect against excessive ant palpation or handling. The thickening of the cuticle varies in different species with amount of ant attendance (Malicky, 1970).

All described lycaenid larvae (even the ones that do not associate with ants) have small, single-celled epidermal glands called pore cupolas (PCOs) (Pierce, 1984). Malicky (1970) described these organs as “perforated cupolas” and noted how the ants “groped and palpated” the surface of the larvae. Malicky also states that these “perforated cupolas” are glandular in nature and release a secretion that acts as a pheromone and may resemble ant brood signals. These signals were later referred to as either adoption (Pierce, 1983) or appeasement (Maschwitz et al., 1985) signals. Complete chemical evidence is still lacking, but it does appear that certain larvae secrete amino acids from the PCOs that mimic ant brood pheromones (Thomas and Elmes, 1993).

The dorsal nectary organ (DNO), another organ of great importance, is located on the dorsum of the seventh abdominal segment in many (but not all) lycaenid larvae. Lycaenid larvae without the DNO are referred to as myrmecoxenous (Kitching and Luke, 1985), while those with the DNO have mutualistic relationships with ants (Fiedler, 1991). A cluster of PCOs is often found around the opening of the DNO (Fiedler, 1991).
Attendant ants palpate the DNO, which releases a sweet, amino-acid rich secretion that is imbibed by the ants (Malicky, 1970; Pierce, 1995). The DNO secretions have been chemically analyzed in several lycaenid species, and the data show variable amounts of carbohydrates and amino acids, depending on the species (Pierce, 1983; 1989).

The third type of myrmecophilous organ is referred to as the eversible tentacle organs (TOs). When present, they are always paired on the dorsal area of the eighth abdominal segment, flanking the DNO. They are able to act independently, responding by eversion to ant stimulation or disturbances (Fiedler, 1991). The TOs remain everted for just a few seconds before they are withdrawn, and they seem to evoke a change in behavior from attendant ants. Ants only respond if they are within a few millimeters of the TOs, and their behavior may be characterized as alertness, alarm, or aggressive posturing (DeVries, 1984; Fiedler and Maschwitz, 1989; Ballmer and Pratt, 1991).

Larvae that have TOs usually have a DNO, and those with only the TO are uncommon (DeVries, 1984). It is more common for a larva to have a DNO and no TO (Fiedler, 1991).

DeVries (1997) has also examined the presence of larvae in the Lycaenidae and Riodinidae families that produce audible, substrate-born vibrations. These larvae “stridulate” against vibratory papillae. In *Thisbe irenea*, these stridulations enhance the larva-ant association (DeVries, 1988, 1990). Travassos and Pierce (2000) found that both larval and pupal calls exist in *Jalmenus evagoras* that act as a predator deterrent as well as maintain the presence of an ant guard.
Life Histories

*Anthene emolus goberus* (Fruhstorfer, 1916) (Lepidoptera: Lycaenidae: Polyommatinae)

The genus *Anthene* Doubleday is found throughout India and the Malay archipelago southeast to New Guinea and the Soloman Islands. It flourishes in tropical Africa, where the highest number of *Anthene* species is found (Corbet and Pendlebury, 1992). The common Ciliate Blue, *Anthene emolus goberus*, is distributed throughout most of peninsular Malaysia, Borneo, Thailand, Singapore, and Sumatra. Two other species, *A. lycaenoides miya* and *A. licates dusuntua*, are found in peninsular Malaysia but are much less common than *A. emolus* (Corbet and Pendlebury, 1992).

The adult *Anthene emolus goberus* has a wingspan of 14-17mm with deep purple-blue coloring in the males and dull brown with purple wing bases in the females. The undersides of the wings are pale brown in both sexes, with a series of bands outlined in white. There is a distinctive orange-crowned black spot on the hindwing above the Cu2 vein, near the margin in space 2 (Fleming, 1975; Corbet and Pendlebury, 1992). Adults are commonly found in tropical lowland rainforest, secondary growth, and forest edges (Fiedler and Maschwitz, 1989; personal observation) where larval food plants are present. Fiedler and Maschwitz (1989) state that “*A. emolus* are especially abundant in disturbed, open habitats in Malaysia where *Oecophylla smaragdina* is one of the most dominant ant species.”

Larval food records include *Nephelium litchi* (Sapindaceae), *Cassia fistula* (Leguminosae), *Heynea trijuga* (Meliaceae) (Fleming, 1975; Corbet and Pendlebury, 1992), and *Saraca thaipingensis* (Caesalpiniaceae) (Eliot, 1980; Fiedler and Maschwitz,
1989). They have recently been found and reared on *Balanocarpus heimii* (Dipterocarpaceae) (personal observation).

Larvae of *A. emolus goberus* are never found in the wild without their obligate attendant ant, *Oecophylla smaragdina* (Eliot, 1980; Fiedler and Maschwitz, 1989; Corbet and Pendlebury, 1992; Fiedler et al., 1996; personal observation). Fiedler and Maschwitz (1989) have shown that *A. emolus* adult females actually use the presence of this ant as an ovipositional cue. Both male and female butterflies frequent areas near *O. smaragdina* nests (personal observation). Additionally, Fiedler et al. (1996) were able to rear *A. emolus* with other ant genera experimentally, but these associations have never been found in nature. Interestingly, *Anthene seltuttus*, an Australian obligate myrmecophile, is only tended by *O. smaragdina*. The facultative myrmecophile in the same genus, *Anthene lycaenoides*, is tended by at least nine different ant genera from four different ant subfamilies (Eastwood and Fraser, 1999).

Larvae develop through four instars (Fiedler and Maschwitz, 1989; personal observation) and they show some general color variation over time and with host plant type and quality. First instars emerge four to five days after eggs have been deposited and then they randomly disperse (Fiedler and Maschwitz, 1989; personal observation). They are pale yellow-green and 1 mm in length. Fiedler and Maschwitz (1989) report observing first instars being picked up and carried into *O. smaragdina* nests and pavilions by ant workers. After two days, first instars moult into second stadia. Second instars may be encountered by ants and are also carried, with equal frequency, to feeding sites or into ant pavilions. A second instar will raise its head and thorax at which point a major ant worker may pick it up and transport it. Adoption by the ant is not guaranteed; larvae
may be ignored or not encountered at all. Attendance by ants is more common for the second instar than the first, but occurs less frequently than attendance of older larvae. The second instar lasts for three to four days, and larvae feed actively by scraping holes in young flushing leaves (Fiedler and Maschwitz, 1989; personal observation). Second instars are usually greenish, but may be shades of red, and begin to develop a brown patch mid-dorsally (personal observation).

Third instars are usually too big to be carried by ants, but some carrying by major workers was observed (Fiedler and Maschwitz, 1989; personal observation). Third and fourth instars eat whole leaves instead of just scraping them. Both of these stages last for three to four days. The third and fourth instars also have developed fully functional DNO and TOs. Larvae secrete glucose-containing droplets from the DNO which are immediately imbibed by the attendant ants. One larva may secrete up to 50 drops in 10 minutes, an amount which represents an important food source for the ants (Fiedler and Maschwitz 1989).

The TOs of third and fourth instars are extruded when larvae are disturbed or when alerted ants approach. The TOs are always withdrawn quickly, but they certainly cause a reaction in the attendant ants. Ants respond to the extension if they are directly tending the larva or are within a 1cm radius. Ants are obviously agitated, and take defensive posturing with their mandibles open (Fiedler and Maschwitz, 1989; personal observation).

The third and fourth instars are usually bright to medium green with a mid-dorsal saddle of brown color. There may be some lateral patterning of brown and green on these larvae. Fourth instars grow to 15-18 mm in length (personal observation).
Fourth instars pupate after three to four days. They wander until a pupation site is found in a somewhat hidden, suitable area. Pupae have been observed near or on ant pavilions and nests as well as in pupal clusters (Fiedler and Maschwitz, 1989; personal observation). Freshly pupated individuals are still visited by attendant ants, and it seems that the DNO is still functional in the prepupal stage (Fiedler and Maschwitz, 1989; personal observation). Pupae are green and girdled to the substrate, with a white scar visible where the DNO was previously located (Fiedler and Maschwitz, 1989; personal observation).

Adult butterflies eclose after seven days, and if they are encountered by *O. smaragdina* ants, they will be killed (Fiedler and Maschwitz, 1989). Additionally, females trying to lay eggs are attacked by nearby ants until actual oviposition commences. It seems that eggs, larvae, and pupae all have a chemical presence that eases aggression but new imagines lack this placating substance (Fiedler et al., 1992).

Parasitoids and predators of *A. emolus* larvae include braconid, ichneumonid, and sphecid wasps as well as tachinid flies (Fiedler and Maschwitz, 1989; Fiedler et al., 1992; personal observation). The rate of parasitism is lower for this species than other myrmecophilous Malaysian lycaenids, presumably due to the close association with the aggressive ants (Fiedler and Maschwitz, 1989). A species of wasp from the Braconidae-Microgasterinae *Apanteles ater* group has been found in *A. emolus* larvae (Fiedler et al., 1992; personal observation). In such parasitism, a wasp larva emerges and pupates in a silken cocoon on the ventral side of the caterpillar, and then a solitary wasp emerges five to six days later. Parasitized *A. emolus* larvae still have functional DNOs and their attendant ants have been observed tending these larvae and drinking the sugary droplets.
Even after the adult parasitoids have emerged, ants will tend the larval carcass for up to four or five days (Fiedler et al., 1992; personal observation).

**Oecophylla smaragdina (Fabricius, 1775) (Hymenoptera: Formicidae: Formicinae)**

Cole and Jones (1948) state that “the genus *Oecophylla* is confined to the Old World Tropics, ranging over the Indo-Malayan, Papuan and Ethiopian regions but does not occur in Madagascar.” The two extant species in this genus are *O. longinoda*, with four varieties, and *O. smaragdina*, with two subspecies and three varieties (Cole and Jones, 1948). *O. longinoda* is found in tropical Africa and *O. smaragdina* inhabits India, Southern Asia, and Northern Australia (Cole and Jones, 1948; Lokkers, 1986). This thesis focuses on *O. smaragdina* of peninsular Malaysia, where they are called weaver ants (Hölldobler and Wilson, 1977, 1990), ferocious red ants (Corbet and Pendlebury, 1992), or keringga (Eliot, 1980; personal communication with locals).

These ants are referred to as weaver ants because of the way they construct their elaborate arboreal nests. They create various sizes and shapes of nests out of most kinds of leaves (Hölldobler, 1983). Worker ants choose leaves to sew together, and form elaborate chains to pull the leaf edges nearer. Linkages of the ants themselves act to ratchet even the toughest leaves together. After the adult workers have grabbed onto leaf edges and pulled them together, other workers shuttle young larvae across the leaf margins, sewing them together with larval-spun silk. Because the larval silk is being used to sew leaves together, the larvae themselves are naked and remain without covering during their larval and pupal stages. This system works because of the highly social nature of these ants. Other workers are devoted to cleaning, feeding, and caring for the developing ant brood. These ants also tend to the highly reproductive queen and keep her constantly fed, either through regurgitations or trophic eggs (Hölldobler and Wilson,
1977). She is able to lay hundreds of eggs a day because of this constant supply of nutrients (Evans and Pierce, 1995).

These weaver ants are one of the most dominant, successful, and highly social species of ants in the world (Hölldobler and Wilson, 1990). They have highly evolved means of both tactile and chemical communication that serve to maintain the colony duties (Hölldobler and Wilson, 1977; Bradshaw, 1981). The dominance of these ants is certainly due to their highly social nature, and they are also one of the most aggressive species of ants in the world (Hölldobler and Wilson, 1990). Mention of the ants’ ferocity has been made even as far back as 1768 by a member of Captain Cook’s party aboard the HMS Endeavor (cited in Musgrave, 1932). He speaks of their industry in nest building and courage in defending the nest, noting the pain he felt when bitten after disturbing a nest and their “revengeful disposition.” These ants will drop down from their arboreal nests to attack potential threats passing below. The powerful mandibles give a painful bite, the effect of which is further increased by the irritating acidic mandibular secretions (Hölldobler and Wilson, 1990).

The cooperation within the colony, aggressiveness of the workers, and high reproductive rate of the queen allow this ant to cover large territories. Individual territories may encompass 21 large, living trees (Hölldobler, 1983) with colonies containing tens and even hundreds of thousands of individuals (Hölldobler and Wilson, 1990).

The aggressiveness and dominance of the ants has also made them useful in biological control. Ant nests were placed in the citrus groves of Southern China as early as 304 A.D., and records show that the ants were very effective at killing citrus pests.
(Hölldobler and Wilson, 1990). They are currently being used to control pests of mahogany and cacao in peninsular Malaysia (Dr. Khoo Kay-Chong and Grace Lim, personal communication). These ants are voracious hunters and attack and kill pests that wander into their territory.

There is a potential problem in using the weaver ants for biological control in certain agricultural cases, however. These ants are often noted for their associations with trophobionts, notably homopterans, mealybugs, and lycaenid larvae (Benzie, 1985). This tending of pest insects (such as scales) would defeat the purpose of these ants as biological control agents. It may even increase the severity of a pest problem, as the ants would defend the scales or other pests instead of killing them (Dr. Yamane and Dr. Khoo, personal communication).

The associations with trophobionts are extremely interesting in this species. In Australia, *O. smaragdina* is associated with one species of parasitic obligate lycaenid, five mutualistic obligate species, and three facultative mutualistic species (Eastwood and Fraser, 1999). Weaver ant nests and surrounding areas are usually covered with a variety of Homopterans, including aphids, scale insects, and plant hoppers as well as associated lycaenid larvae. Workers constantly tend and “milk” these insects for their nutritious secretions. Workers have been observed carrying larvae of *A. emolus* to more nutritious, young leaf material, presumably to improve the quality of these secretions (Fiedler and Maschwitz, 1989). The shuttling of larvae to “grazing sites,” and the acquisition of a “standing flock” of other sugar-producers, seem rather analogous to these ants tending to livestock.
CHAPTER 2
ANT MORPHOLOGY AND CASTE DISTINCTION

Introduction

Weaver ants of the genus *Oecophylla* represent a highly organized group of social insects. In this study, *O. smaragdina* were specifically examined. Several authors have examined the castes of this species in Australia (Cole and Jones, 1948; Hölldobler and Wilson, 1977, 1990; Chapuisat and Keller, 2002). This study examines the two castes as they occur in peninsular Malaysia.

Examination of ants was necessary to establish the presence of two morphologically distinct castes and these results were used later in this thesis to examine the behavioral differences between the castes of ants. Minor ants were not often observed, as they reside primarily in the ant nest. Major ants forage and defend the nest and are highly visible. There was a possibility that major ants actually exist in two castes, but there are no morphological data to support this. Whether there is a temporal caste distinction based on age remains to be shown conclusively (Wilson, 1976). Other authors have noted that older major ants reside in “barrack nests” and defend the main nest from attacks (Cole and Jones, 1948; Hölldobler and Wilson, 1990). Data show no difference in ant size other than that between the major and minor ants.

In peninsular Malaysia, major ants are approximately 10mm in length and are reddish in color. They have long legs and antennae, and construct and defend the nest in addition to foraging. Minor ants are smaller at 5 mm in length, with shorter legs. The queen is a large, 20 mm green-bodied insect that never leaves the nest except to mate.
The males are smaller than major ants and black in color. Males die after mating with virgin queens. According to previous studies, major and minor ants of this species from Australia are notably smaller than from Malaysia (Chapuisat and Keller, 2002).

**Methods**

Three *Oecophylla smaragdina* ant nests were collected at FRIM, Selangor, peninsular Malaysia on November 13, 21, and 23, 2002. Nests were taken from three different types of trees: *Balanocarpus heimii* (Dipterocarpaceae), *Artocarpus heterophyllus* (Moraceae), and *Dyera costulata* (Apocynaceae). Common names for these trees are cengal, jackfruit, and jelutong, respectively. Nests were from different populations of ants and no overlap existed between foraging territories of these nests. Our assumption is based on the fact that no foragers were seen in between nest areas and there was considerable distance (over one km) between nests.

Nests were collected by clipping the small branches of the trees supporting the nests with long-handled orchard pruners. Fallen nests were placed immediately into individual garbage bags. Bags were labeled and stored in a freezer overnight to kill the ants. All ants (including worker castes, males, larvae, pupae, and queens) were preserved in 70% alcohol. Other insects present in the nests were also preserved in alcohol. All ants were later removed and counted.

One hundred ants from each nest were randomly removed from alcohol with forceps. Nest 1 is the only exception, as all 115 ants present in the nest were surveyed. Ants were then individually measured in a 4 x 4 cm glass dissecting block with a 1 cm depression. Of this depression, a quarter was filled with small glass shards and 75% undenatured alcohol. Ants were submersed in the glass/alcohol mixture and manipulated with forceps until a clear, fixed view of the head was possible.
Two measurements were taken from each ant, using the scale in the ocular piece of the microscope. The first measurement was taken across the middle of the eyes (the widest point of the head). The second measurement was that of the length of the left mandible from the point of head insertion to the tip (Fig. 2-1). All measurements were calibrated to a millimeter scale. A glass slide millimeter scale was used to test the accuracy of the conversion from the ocular scale bar to millimeters. The millimeter slide was used after every three ants were measured to make sure that the scale and magnification remained accurate. Measurements were later put into histograms to check for distribution and bimodality of castes using Minitab 13.

Figure 2-1. Measurements were taken across the eyes to determine head width of each sampled *Oecophylla smaragdina* ant. Measurements also were taken along the length of the left mandible from the point of insertion to the tip.

**Results**

Histograms show a clearly bimodal distribution of ant castes in all three nests, with morphological differences denoting a major and minor caste. The mean head width for major workers is 1.737 mm ± 0.124 (mean ± SD), and for minor workers it is 1.145 mm ± 0.072 (Fig. 2-2). An upper limit for head width of minor workers is 1.4 mm. The mean
left mandible length is $1.157 \text{ mm} \pm 0.087$ for major workers and $0.626 \text{ mm} \pm 0.07$ in minor workers (Fig. 2-3).

Figure 2-2. Histogram of *Oecophylla smaragdina* head width. Data are pooled from three separate nests and show a bimodal distribution of ants.

Figure 2-3. Histogram of *Oecophylla smaragdina* mandible length. Data are pooled from three separate nests and show a bimodal distribution of ants.

After measurements were taken, all ants were removed from nests and counted (Table 2-1). It is likely that Nest 1 was a barracks nest, due to its small size. In large nests like that of Nest 2, major and minor workers are equal in numbers. This large,
conspicuous nest also had 13 queens inside. Nest 3 was smaller and possibly recently founded by the one queen found inside.

Table 2-1. Total numbers of *Oecophylla smaragdina* sampled from three nests collected at FRIM, peninsular Malaysia.

<table>
<thead>
<tr>
<th>Nest</th>
<th>Total number of worker ants</th>
<th>% Majors</th>
<th>% Minors</th>
<th># of queens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>115</td>
<td>60.87</td>
<td>39.13</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3858</td>
<td>49.01</td>
<td>50.98</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>337</td>
<td>62.01</td>
<td>37.98</td>
<td>1</td>
</tr>
</tbody>
</table>

The polydomous nature of these ants allows them to patrol large areas that encompass many trees. The nests that were sampled represent a range of sizes and, perhaps, of functions as well. It is interesting to note that Nest 2, which was the largest and also held the most brood and queens, had a roughly equal ratio of major to minor workers. Barrack nests are full of majors and represent the first line of defense for a multi-tree colony. Majors in other nests can forage for food for the queen and for developing brood. Minor ants have no real function in barrack nests as they are not large enough to play a role in defense. They tend scale insects inside the nests and regurgitate secretions for major workers. In Australia, both major and minor workers in barrack nests have been found to be aged individuals (Hölldobler, 1983). The identification of two distinct morphological castes allowed us to make caste-specific definitive behavioral observations.
CHAPTER 3
FIELD OBSERVATIONS OF ANTS AND LARVAE

Introduction

Larvae of *Anthene emolus* have been observed in association with weaver ants, *Oecophylla smaragdina* (Fiedler and Maschwitz, 1989). This relationship has been deemed obligate as *A. emolus* larvae are never found without the ants in attendance. It is believed that the ants protect the vulnerable lycaenid larvae from parasitoids and predators and thus ensure their low mortality. The larvae, in return, secrete droplets of nutritious secretions that the ants imbibe.

The level of ant-lycaenid association at different caterpillar life stages is uncertain. Young larvae have been observed being picked up and carried by ants. It also appears that ants prefer to tend the larger caterpillars of late instars. The main question, however, is whether the different castes of ants differentially tend larvae. Historical records of this symbiosis do not distinguish between the major and minor castes of ants. This study aims to elucidate the behavior of the ant castes when *A. emolus*, a significant food source, is present. The elucidation of this relationship will give greater insight into the relationship not only between the ants and larvae, but between the two castes of ants.

Methods

Behavioral Observations (in the Field)

All field observations were taken inside the 1600-ha Forest Research Institute of Malaysia (FRIM) in Selangor, peninsular Malaysia. The study site was inside the plant
nursery, in a 0.25-ha plot containing ~100 Balanocarpus heimii trees. All trees were approximately 2m in height.

Observational data were recorded from November 2002-April 2003. Time, weather, and temperature were recorded. Observations were conducted in morning sessions (9 AM – 12 PM) and in the afternoon (2 PM – 5 PM). After all observations were finished for the respective session, the following information would additionally be taken: the distance of the tending activity from the closest ant nest, the presence of Homoptera or other insects, and the length of the lycaenid larvae. Larval length was taken with a small field ruler and was later used to correlate length to instar number (Table 3-1).

Table 3-1. Correlation between instar of A. emolus and body length (mm).

<table>
<thead>
<tr>
<th>N</th>
<th>Instar</th>
<th>Length (range in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L1</td>
<td>1-2</td>
</tr>
<tr>
<td>27</td>
<td>L2</td>
<td>3-5</td>
</tr>
<tr>
<td>25</td>
<td>L3</td>
<td>6-8</td>
</tr>
<tr>
<td>15</td>
<td>L4</td>
<td>9-15</td>
</tr>
</tbody>
</table>

Before taking the behavioral observation data, fifteen minutes were allowed to elapse to allow the ants to acclimate to the presence of the observer. Ants act very defensively towards the observer due to the disturbance caused to the environment. They may even leave the area and hide. After the acclimation time passed, observations were taken every minute for ten minutes.

Observations focused on the behavior of ants in relation to a lycaenid larva. One to three larvae were monitored during each ten minute period, given that the distance between larvae was not too great. Every minute, observations were taken regarding the presence or absence of ants, the number of ants from each caste, and behavior of ants of each caste. Ant behavior was categorized in nine ways: tending larva, carrying larva,
trying to tend larva (blocked), tending Homoptera, self-grooming, allogrooming (grooming another ant, includes trophallaxis), ignoring larva, alarm, or foraging. These data were recorded when ants were touching a larva or were within 2 cm from it. The main behavior observed during the minute of time was recorded. Only behavioral activities that lasted for 30 seconds or more of the one-minute observational period were recorded. This means that a one-minute period may show 0.5 minutes of two different behaviors. Behaviors were later scored to the amount of time they occurred out of ten minutes. Occurrence of larval tending was the only behavior considered for analysis. Frequencies of larval tending were then compared between the major and minor ant castes at each of the four instars.

**Behavioral Observations of (Captive-Laid Eggs Placed in the Field)**

*Anthene emolus* butterflies were caught on the roof of the Natural Forest Division, FRIM, approximately 16 m off the ground. This location was on the forest edge and allowed access to the canopy of several forest trees. Butterflies were kept in two 25 x 25 x 30-cm plastic boxes with a circular hole at the top. The hole was secured with a sleeve of fine nylon mesh, to allow access into the box without allowing the butterflies to escape. Butterflies were fed daily with a 50% sugar water solution from a cotton ball in a standard sized glass petri dish. Host plant cuttings with new growth wrapped in wet cotton in small plastic water bottles were placed in the boxes. Four *O. smaragdina* were present in each box to act as ovipositional cues. The sides of the boxes were lined with clear plastic transparency sheets taped at the top and sides. The bottoms of the boxes were lined with plain white paper.

Eggs that were easily removed were collected from the boxes when they were laid on leaves, petri dishes, transparency sheets, or other materials in the box. Eggs were
transferred to standard sized glass petri dishes with lids for 3-4 days, i.e. until they were about to hatch.

Four batches of eggs (still on the plastic sheet substrate) were placed in each of three field locations in proximity to *O. smaragdina* nests. They were scotch-taped on leaves around ant nests in places where it was conceivable that female *A. emolus* would lay eggs. The four egg batches were taped to a variety of young and old leaves as well as the bark of the tree in which the ant nest resided. The locations of the ant nests and taped egg clutch sizes are summarized in Table 3-2. Locations were checked daily.

Table 3-2. Locations in FRIM, peninsular Malaysia where *A. emolus* eggs were taped to foliage near *O. smaragdina* nests.

<table>
<thead>
<tr>
<th>Site name</th>
<th>Location of trees where nests occur</th>
<th>Number of eggs/clutch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roof of FRIM building</td>
<td>16 m high in forest edge</td>
<td>8, 10, 91, 125</td>
</tr>
<tr>
<td>Fish Pond</td>
<td>disturbed area at forest edge</td>
<td>3, 7, 42, 60</td>
</tr>
<tr>
<td>Fruit Tree Arboretum</td>
<td>collection of planted fruit trees</td>
<td>25, 54, 68, 108</td>
</tr>
</tbody>
</table>

Results

**Behavioral Observations (Field Conditions)**

There were definite behavioral differences between the major and minor castes of *O. smaragdina* in the natural field conditions when *A. emolus* caterpillars were present. Major workers tended all *A. emolus* larvae, regardless of instar, at a significantly higher rate than minor workers tended caterpillars (*p*=0.0001, 95% CI, Mann-Whitney Test) (Fig. 3-1). Major workers additionally tended 4th instars more than they tended 2nd instars (*p*=0.002) (Table 3-3).

Individual major workers were also observed carrying 2nd instars into ant pavilions or to young flushing leaves on three occasions (Fig. 3-2). On five other occasions, major workers carried late instars away from an approaching parasitoid. But three times, as the
larvae everted their TOs, several major workers responded by picking up and pulling the larvae apart (Fig. 3-3).

Figure 3-1. Box plot of frequencies of major and minor ants tending second instars. Means are indicated by red circles. One outlier exists in the minor ant data. This minor ant tended a larva for 8 minutes while no majors were present.

Table 3-3. Individual *A. emolus* larvae were observed for 10-minute periods.

<table>
<thead>
<tr>
<th>Ant <em>Anthene emolus</em> caterpillars</th>
<th>Ant Majors tending caterpillars (mean min./10 min.)</th>
<th>Ant Minors tending caterpillars (mean min./10 min.)</th>
<th>Ant Minors trying to tend caterpillars (mean min./10 min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second instar</td>
<td>5.86±3.13</td>
<td>2.32±2.44</td>
<td>0.40±0.74</td>
</tr>
<tr>
<td>nL2 = 27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third instar</td>
<td>6.98±3.69</td>
<td>1.25±3.04</td>
<td>0.40±0.77</td>
</tr>
<tr>
<td>nL3 = 25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fourth instar</td>
<td>9.59±3.76</td>
<td>0.72±2.00</td>
<td>0.70±1.50</td>
</tr>
<tr>
<td>nL4 = 15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two-sample t-tests</td>
<td>p=0.002</td>
<td>p=0.0005</td>
<td>p=0.61</td>
</tr>
<tr>
<td>Second vs. Fourth instars</td>
<td>nL2 = 27</td>
<td>nL2 = 26</td>
<td>nL2 = 27</td>
</tr>
<tr>
<td></td>
<td>nL4 = 15</td>
<td>nL4 = 14</td>
<td>nL4 = 15</td>
</tr>
</tbody>
</table>

The average number of minutes (in 10-minute period) a larva was tended by at least one ant caste member is given. All ants are from the same *Oecophylla smaragdina* colony. Two-sample *t*-tests compare the difference in ant tending between the second and fourth instars. Total n=67.
Figure 3-2. A major worker of *O. smaragdina* carrying a young *A. emolus* larva.

Minor workers were never observed carrying larvae. Minor workers were observed trying to tend larvae, but were kept away by major workers (Table 3-3). A total of 67 larvae was observed, and minors were blocked from tending 33.3% of the 4th instars, 20% of the 3rd instars, and 22.2% of the 2nd instars. Major ants were particularly aggressive against minor ants that tried to tend 4th instars that were already being tended by major workers. Minors were able to tend older larvae only in the few cases that majors were not present (Fig. 3-4). Many observations were made with more than one major ant tending a larva (Fig. 3-5), but only twice were majors and minors tending the same larva.
Figure 3-3. Majors reacting to 4th instar *A. emolus* larva (and later killing it).
Figure 3-4. A minor worker was able to tend a late instar *A. emolus* caterpillar when no major workers are present.
Several major workers may tend an *A. emolus* caterpillar together. Note that no minor workers are present.

**Behavioral Observations of (Captive-Laid Eggs Placed in the Field)**

No *A. emolus* caterpillars resulted from eggs laid on plastic that were placed at the various field sites. The eggs, the plastic the eggs were laid upon, or the entire leaf where the plastic was taped, were removed from every location they were placed, presumably by *O. smaragdina* ants. Naturally-laid eggs evoke no response from ants and larvae were able to hatch without predation by *O. smaragdina*. But in this instance, the ants may have detected the other odors associated with plastic and tape and instead treated the eggs as intruders to be removed (Fig. 3-6).
Figure 3-6. The taped egg batch (circled in black) on a ficus leaf on the roof of FRIM, before its removal by ants. The ant nest is located out of view, under the taped egg batch.
CHAPTER 4
LARVAL FITNESS AND MORTALITY AS AFFECTED BY ANT CASTE

Introduction

Laboratory experiments are important in elucidating the symbiotic relationship between lycaenid larvae and attendant ants. Cushman et al. (1994) showed that the southeast Australian lycaenid *Paralucia aurifera* larvae are significantly heavier, develop faster, exhibit shortened pupation time, and have larger adults when larvae are reared with ants present. Attendant ants protect the lycaenid larvae and larvae are able to spend more time feeding. Not all lycaenid species show such dramatic results when experimentally raised with and without ants. Studies of *Hemiargus isola* in Arizona show that although larvae may be bigger when ant-tended, there are other physiological costs due to the significant amount of nutrients secreted for the ants (Wagner, 1993; Wagner and Martinez del Rio, 1997). These larvae, however, are able to compensate and even overcompensate for such losses (Wagner, 1993). Increased larval weight may in fact be due to the ants reducing the energy expenditure of tended larvae (Wagner and Martinez del Rio, 1997).

Other studies show that ant-tending may be more costly to female larvae (Fiedler and Hölldobler, 1992) or that larvae take a longer time to develop (Robbins, 1991). Baylis and Pierce (1992) have shown that Australian *Jalmenus evagoras* larvae which are heavily tended by ants show reduced pupal weight versus non-tended individuals. Although this lowers the intrinsic rate of population increase in lycaenid species, authors still agree that myrmecophily represents an evolutionarily stable strategy.
It has been established that *Oecophylla smaragdina* protect *Anthene emolus* larvae from parasitoid and predator attack (Fiedler and Maschwitz, 1989; personal observation). This present study examined other ways that ant tending, and specifically tending by different castes, affect larval fitness, survivability, and adult weight.

**Methods**

All experiments were conducted in clear plastic rectangular boxes (22.5 x 13.5 x 8.0 cm) with tight-fitting lids in the FRIM Insectarium Laboratory at 24-25˚C and approximate 12L:12D cycle. All boxes contained a similar amount of young leaf flush of *Balanocarpus heimii* taken from the FRIM nursery. Plant material was replaced every other day. The ends of the plants were cut at an angle and wrapped in cotton wadding soaked in deionized water. Each box had the same amount of wet cotton to keep humidity relatively constant. The bottoms of the boxes were lined with plain white paper which was changed every other day.

One large clutch of *A. emolus* eggs was collected from the transparency sheet of the captive female butterfly box (see Methods in Chapter 3). The clutch was cut out and placed in a covered glass Petri dish for three days. Eggs were separated from the clutch with a small camel hair brush, and two undamaged eggs were placed on young leaves in each box.

Tests included four treatments, each ran concurrently and replicated nine times:

- Treatment A - contains four major ants
- Treatment B - contains four minor ants
- Treatment C - contains two major and two minor ants
- Treatment D - contains no ants.
All ants used were collected from the same colony in the Fruit Tree Arboretum, FRIM. This colony encompassed several trees and contained many nests and pavilions. Nests were individually clipped from their branches and kept indoors in large, sealed plastic bags to supply ants for the experiment. The number and caste of ants was kept constant in each box. If an ant died during the course of the experiment, it was removed and replaced with an ant of the same caste from the bagged nest. Using ants from the same colony was essential, as ants would otherwise fight and kill each other. When the ants in the bag were dead or depleted, another nest from the same colony would be clipped from the tree and kept in a bag. Ants were monitored and replaced daily. Ants were not given any external food source once they were placed in a bag.

Larval fitness was recorded for each treatment box by measuring larval length and also the time spent as larvae. Larval lengths were recorded daily and graphed as growth (mm) over time (days) in MS Excel. Overall mortality as well as the number of butterflies able to eclose successfully were recorded. Larvae were not weighed because removing larvae disturbs the attendant ants too much. If larvae died during the experiments, they were removed from the box and kept in alcohol.

Adult butterflies were killed in the freezer, then placed in individual glass vials of silica. They were dried for 2 hours in a 104 °C oven, allowed to cool for 15 minutes, and then weighed on an electronic scale. Dry weights of eclosed adults were analyzed in Minitab 13.

**Results**

Larvae developed an average of 1.15 days more quickly when they were tended by both castes of ants (Treatment C) than when they were not tended by ants (Treatment D). Larvae from Treatment B (only minor ants included) and Treatment C developed the
most quickly, at a total of 11.75 and 11.77 days, respectively. The total amount of time spent as pupae was similar for all treatments. The average number of days each larval stage lasted is summarized in Table 4-1.

Table 4-1. Summary of statistics for experiments with ant treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average # days spent as larva</th>
<th>% eclosion of larvae n=18</th>
<th>Average adult dry weight (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12.60</td>
<td>50.0%</td>
<td>0.0193±0.0038</td>
</tr>
<tr>
<td>B</td>
<td>11.75</td>
<td>33.3%</td>
<td>0.0124±0.0032</td>
</tr>
<tr>
<td>C</td>
<td>11.77</td>
<td>66.7%</td>
<td>0.0103±0.0033</td>
</tr>
<tr>
<td>D</td>
<td>12.92</td>
<td>55.6%</td>
<td>0.0097±0.0028</td>
</tr>
</tbody>
</table>

Treatment A contained four major ants, Treatment B contained four minor ants, Treatment C contained two major and two minor ants, and the Treatment D contained no ants.

Average larval growth rates are shown in Figure 10 for each treatment. Analysis of data showed an exponential distribution of larval growth rate, and an exponential trend line was added to the daily measurements of larval length (Fig. 4-1). Treatment B, where only minor ants tended larvae, showed the steepest slope, representing the fastest growth rate. The Treatment C replicates, where both major and minor ants were present, showed the slowest growth rate.

Although each treatment was replicated nine times and with two larvae per box, no more than twelve butterflies per treatment eclosed. The small sample size puts this study into a “pilot” study category. The lowest percentage of eclosion occurred in the treatment of larvae reared with only minor ants. Only six butterflies emerged after starting with 18 larvae (33.3% survived). Nine butterflies emerged from the treatment with majors only (50% survival), ten from the treatment with no ants (55.6%), and twelve from the control with both castes (66.7%).
There were no significant differences in butterfly dry weights as a result of the experimental rearing conditions of the larvae (Table 4-1). One of the larvae raised in the absence of ants developed a fungal infection around the DNO (Figure 4-2). It pupated the next day and eclosed as a normal butterfly. Two pupae died from an apparent virus in the treatment with only minor ants.

Figure 4-1. Average growth rates of *A. emolus* larvae when reared with the various ant treatments. An exponential trend line (in heavy black color) has been fitted to each graph and trend line slopes are labeled. A) Treatment A contained four major ants. B) Treatment B contained four minor ants. C) Treatment C contained two major and two minor ants. D) Treatment D contained no ants.
Figure 4-2. Final instar from Treatment D (reared with no ants) with a presumed fungal infection around the dorsal nectary organ (DNO).
The results confirm the presence of morphologically distinguishable major and minor ant castes in *Oecophylla smaragdina*. Behavioral differences in the ant castes were evident in the field. However, the effect that different ant castes have on larvae of *Anthene emolus* is less clear.

As minor ants do not spend a significant amount of time independently tending larvae in the field, the results of Treatment B (rearing larvae with only minor ants) have little bearing on a natural situation. Larvae reared under this condition grew the most quickly, but resulted in the fewest eclosed butterflies.

Larvae raised with both castes of ants grew at a slow rate but yielded the highest number of butterflies. These larvae grew at a slow overall rate but still pupated a day more quickly than larvae reared with no ants. Larvae raised with both castes of ants might have grown more slowly because the ants constantly taxed them for secretions. However, they might have fed more or digested food more efficiently to compensate for this nutrient loss, and this has resulted in a higher number of successfully eclosed butterflies. Tended larvae may also conserve energy in the field, as major ants may carry them around the host plant, but this was not evident in lab studies.

As the field data show that major ants spend the most time tending larvae and do not allow minors to tend larvae, it would follow that Treatment A (only majors) and Treatment D (both majors and minors) would yield similar results. Larvae from both experiments grew at comparable rates, but the treatment with both ant castes produced
larvae that pupated one day earlier than larvae reared with only major ants. The control additionally yielded a higher number of successful eclosions. This would indicate that the minor ants have a significant effect on larval development. Larvae from Treatment B did pupate quickly as well, but only a small number eclosed. More trials need to be run to evaluate this situation, as the sample size was low.

In field observations, aggression by major workers against minor workers defies the basic tenets of cooperation that are essential to social insect societies. Trophallaxis is commonly observed in this species (in both the lab and the field), between both major and minor individuals, so the evidenced exclusion of a food source is aberrant behavior. The dominance of the major workers and exclusion of minors was commonly observed in the field, although an explanation has yet to emerge.

The three larvae that were killed by major workers in the field may be a rare behavioral anomaly. The presence of threat (e.g., a parasitoid or the observer) may have caused the TOs to be everted by the larvae. The brief release of alarm pheromone may have affected the major workers in attendance, and it is perhaps the behavior of these ants that served to recruit the rest of the ant guard. The overzealousness of these ants in impacting jaw closure served to puncture the cuticle and kill the larva. Interestingly, the majors were able to synchronize their movements and jointly carry the dead larva away to a pavilion.

The evolutionary newness of this symbiosis may be at the root of such questions and the apparent overlap in the major and minor ant tasks. Such an advanced, obligate relationship takes time to develop and will continue to develop under changing conditions. The aberrant behavior may represent an evolutionary “kink” in this system.
Minor workers are rarely seen outside the nest, except when lycaenid larvae are present. They do, however, tend scale insects and ant brood. Major workers are responsible for defending their domain against intruders, but they have allowed and encouraged *A. emolus* larvae to feed on the best plant shoots. Majors also secure most of the food resources for the nest (Hölldobler and Wilson, 1990), and thus it makes sense that they collect the lycaenid secretions. Both major and minor castes have valid claims to gather lycaenid secretions, and in this instance it seems that the major’s “might makes right.”

An age-based ant caste may exist, beyond the presence of major and minor castes. Ant colonies send older individuals to defend the nest (Hölldobler and Wilson, 1990), and it may in fact be these older ants that are collecting some of the secretions from *A. emolus* larvae. Older, minor workers that are sent from the inner nest may encounter larvae and try to tend them, but they encounter aggression from major workers. By dissecting the fat bodies, we could tell the relative ages of the ants found tending lycaenid larvae.

Further field work needs to be done to ensure that this is not a localized phenomenon. Volatiles from TOs should also be collected and used in bioassays to elicit behavioral responses from both castes. These insects offer an opportunity to study symbioses in a natural setting, and perhaps it will lend insight into the development of other species’ symbioses, inter-specific communication, and the allocation of resources.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Emily Heffernan was born July 29, 1977, in Syracuse, NY. She grew up with three brothers, wonderful parents, and an ever-changing assortment of pets. She graduated from high school in NY a semester early, and spent time in Denmark with one of her brothers. After spending two years at Syracuse University, first as a linguistics and then a biology major, she transferred to New College at the University of South Florida in Sarasota, Florida. It was there that her passion for conservation and an interest in insects began. After completing her bachelor’s degree in biology, she spent a year traveling around the world. Beginning with old friends in Denmark, she visited Oman, Sri Lanka, Indonesia (including Bali and Komodo Islands), Thailand, Malaysia, and Singapore. She taught English in a small, village school in Bali for several months. The majority of her free time was spent snorkeling, trekking, and rock-climbing in Southeast Asia.

She returned to Malaysia under a Fulbright Fellowship, to conduct her M.S. research. She was later accompanied by Justin Saarinen. They will be married in April 2005, and plan to continue traveling and conducting scientific work in the field of conservation. They are always accompanied by Emily’s other best friend, her dog Tex.