



# Association between the African lycaenid, *Anthene usamba*, and an obligate acacia ant, *Crematogaster mimosae*

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The African lycaenid butterfly, *Anthene usamba*, is an obligate myrmecophile of the acacia ant, *Crematogaster mimosae*. Female butterflies use the presence of *C. mimosae* as an oviposition cue. The eggs are laid on the foliage and young branches of the host plant, *Acacia drepanolobium*. Larvae shelter in the swollen thorns (domatia) of the host tree, where they live in close association with the acacia ants, and each larva occupies a domatium singly. *Anthene usamba* are tended by ants that feed from the dorsal nectary organ at regular intervals. Larvae also possess tentacle organs flanking the dorsal nectary organ and appear to signal to ants by everting these structures. Larvae were observed to spend most of their time within the domatia. Stable isotope analysis of matched host plant–ant–butterfly samples revealed that *Anthene usamba* are  $\delta^{15}\text{N}$  enriched relative to the ants with which they associate. These data, based on the increase in  $\delta^{15}\text{N}$  through trophic levels, indicate that the caterpillars of these butterflies are aphytophagous and either exploit the ant brood of *C. mimosae* within the domatia, or are fed mouth to mouth by adult workers via trophallaxis. This is the first documented case of aphytophagy in African *Anthene*. Pupation occurs inside the domatium and the imago emerges and departs via the hole chewed by the larva. The adult females remain closely associated with their natal patch of trees, whereas males disperse more widely across the acacia savannah. Females prefer to oviposit on trees with the specific host ant, *C. mimosae*, an aggressive obligate mutualist, and avoid neighbouring trees with other ant species. Adult butterflies are active during most months of the year, and there are at least two to three generations each year. Observations made over a 5-year period indicate that a number of different lycaenid species utilize ant-acacias in East Africa, and these observations are summarized, together with comparisons from the literature. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, ●●, ●●–●●.

**ADDITIONAL KEYWORDS:** *Acacia drepanolobium* – mutualism – Myrmicinae – parasitism – Polyommatae – Kenya.

## INTRODUCTION

Associations between ants and lycaenids provide classic examples of complex symbioses. Lycaenid butterflies are conspicuous and have evolved diverse relationships with ants that range from mutualism to parasitism (Hinton, 1951; Atsatt, 1981b; Pierce &

Mead, 1981; Pierce, 1983; Cottrell, 1984; Fiedler & Maschwitz, 1989), including both trophobiosis (exploiting ants by begging and being fed liquid food regurgitated by ants) and kleptoparasitism (exploiting ants by stealing and consuming ant brood) (Pierce *et al.*, 2002). In a typical interaction, ants tend the lycaenid caterpillar and are rewarded with secretions from the larva's dorsal nectary organ, whilst the caterpillar gains protection from parasitoids and predators. Highly specialized ant–lycaenid

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associations appear to be especially rich in southern Africa and Australia, regions in which ant-associated lycaenid diversity is well documented to be high (Clark & Dickson, 1971; Common and Waterhouse, 1981; Henning, 1983; Braby, 2000). Most of the work on ant–lycaenid interactions in Africa has been conducted in southern Africa (Clark & Dickson, 1971; Heath *et al.*, 2008; but see also Lamborn, 1913; Farquharson, 1921; Jackson, 1937).

Eastern Africa includes large areas of savannah dominated by *Acacia* spp. (Beentje, 1994; Shorrocks, 2007). It should be noted that we use *Acacia s.l.* here, as the placement of African species into different genera is still under dispute (Moore *et al.*, 2010). These members of the legume superfamily are well known to be among the major food plants of lycaenids (Clark & Dickson, 1971; Pierce, 1985; Larsen, 1991; Woodhall, 2005). This region, including the Great Rift Valley, encompasses regional centres of endemism and global biodiversity hotspots (Bennun & Njoroge, 1999). One African ant-acacia, *Acacia drepanolobium*, is a widespread species covering large areas of savannah in monospecific stands. Previous studies have documented four principal ant mutualists of *A. drepanolobium* in Laikipia, northern Kenya, and their patterns of host tree occupancy: *Crematogaster mimosae*, *C. nigriceps*, *C. sjostedti* and *Tetraponera penzigi* (Young, Stubblefield & Isbell, 1997; Palmer, Stanton & Young, 2002). A number of facultative ant associates are also known, but are comparatively rare (Kuria, 2006). The host plant provides housing for mutualist ants in the form of hollow thorns that act as domatia for the ant brood, and food rewards via extrafloral nectaries. The four ants vary in their degrees of mutualism with the host and their abilities to colonize plants, invade occupied plants and defend their host against invasion by other ants (Young *et al.*, 1997; Stanton *et al.*, 1999; Palmer *et al.*, 2000, 2011; Martins, 2010; 2013; Stanton & Palmer, 2011). Three of the ant mutualists, *C. mimosae*, *C. nigriceps* and *T. penzigi*, are phytoecious, residing only in the domatia provided by the plant and never found living separately from the acacias, whereas *C. sjostedti* is sometimes found free-living. This region is also rich in lycaenid butterflies (especially ‘blues’ in the Polyommatainae), including members of the genus *Anthene*, whose larvae are known to associate with ants (Jackson, 1937, 1947; Libert, 2010). One species, *Anthene emolus*, is known to associate with the aggressive ant species *Oecophylla smaragdina* in Asia and Australia (Fiedler & Maschwitz, 1988; Braby, 2000; Saarinen, 2006). In Africa, no *Anthene* have been recorded with *Oecophylla*, although species of *Euliphyra* are obligate associates of this ant.

In systems with more than one potential ant associate available to lycaenids, selection should favour

lycaenids that associate with ants that are better tenders or more closely associated with their host plant, as has been found in the case of Australian species, such as *Jalmenus evagoras* feeding on acacias (Pierce & Elgar, 1985; Thomas, 1985; Pierce & Eastal, 1986; Pierce *et al.*, 1987) and *Ogyris amaryllis* on mistletoes (Atsatt, 1981a). The focus of this study, *Anthene usamba* (previously classified as *A. hodsoni*; Libert, 2010), has been documented as occurring within the biogeographical range of whistling thorn acacias, *A. drepanolobium*, and, although it has been recorded to associate with ants (Larsen, 1991), no detailed work on the interaction between the two has been performed. This study is the first to tackle in detail the interactions between an ant–plant and ant–lycaenid symbiosis in East Africa through both behavioural ecology and stable isotope methods. The goals of this study were: (1) to describe the association between *A. usamba* and the ant-acacias, including general observations of the interactions between the caterpillars and ants, and, given the different species of ant known to associate with the acacia, (2) to test the oviposition preference with respect to different ant species and to investigate more closely how female *A. usamba* respond to the presence of different ants, and (3) to use stable isotopes to explore the interactions between the acacias, ants and lycaenids in an East African system in which they have not been applied previously.

#### STUDY SITES

This research was conducted in Kenya between May 2005 and November 2011. In the savannahs of this region, *A. drepanolobium* is a common feature of the vegetation, forming large, dense, near-monospecific stands on high-altitude grasslands with black-cotton clay soils. The sites used during the study were located in Kajiado North County and Laikipia County. Two sites were located in Kajiado: ‘Kitengela’ (Oloorsirkon Location) (36°49'E, 1°23'S; 1660 m elevation) and Ngong Hills (36°38'E, 01°26'S, 2000 m elevation); one site was located in Laikipia: Suyian Ranch (36°42'E, 0°32'N; 1820 m elevation). Experiments and observations were conducted at multiple locations in these areas during and after the main rains. The Kitengela (Oloorsirkon) and Ngong sites occur within the bimodal annual rainfall pattern experienced in this habitat through the action of the Inter-Tropical Convergence Zone, with the ‘long rains’ beginning in April and running through May into early June, and the ‘short rains’ in November–December (Bennun & Njoroge, 1999; Shorrocks, 2007). The Suyian (Laikipia County) site has a trimodal pattern of rainfall, with a smaller peak of rainfall in August in addition to the April and November rains.

The Kitengela (Oloorsirkon), Ngong and Suyian study sites have three of the four most common ant mutualists of *A. drepanolobium* in abundance: *C. mimosae*, *C. nigriceps* and *T. penzigi*. Populations of *A. usamba* occur at all of these sites, where they are thought to be associated with acacias (Larsen, 1991). Scale insects (*Stictococcus* sp.) occur in association with the ant *C. mimosae*. These are sessile scales that appear to be obligately associated with this ant (Hocking, 1970; Richard, 1976; D. J. Martins, unpubl. data).

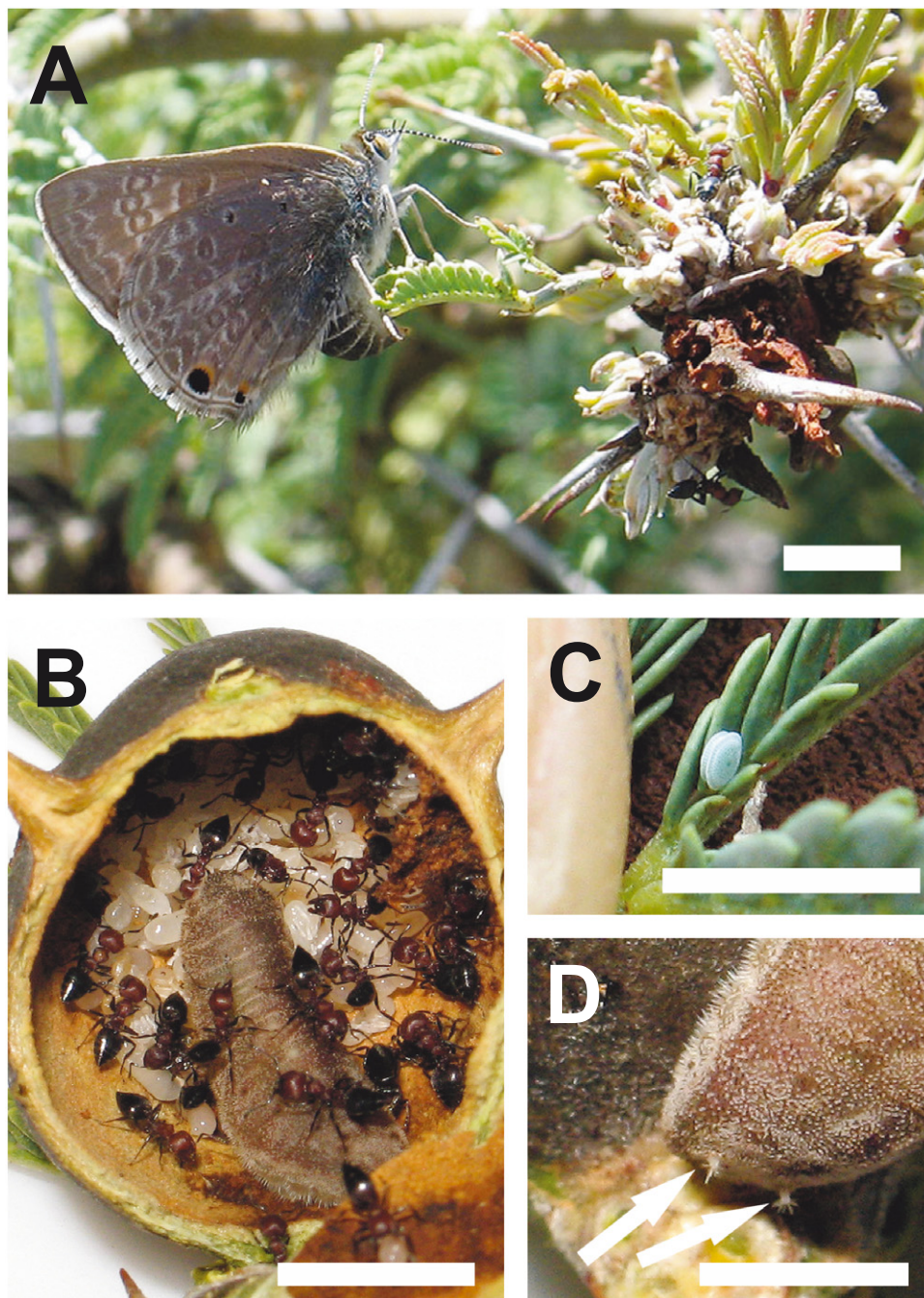
## METHODS

Observations of larvae and adults of *A. usamba* were made both in the wild and with captive individuals. We censused adult lycaenids along transects, and noted the dates on which they were active. This was performed monthly through the study period (January 2005 to November 2011). We looked for larvae on stands of trees in which adult females had been observed circling (Fig. 1). Domatia containing larvae were identified by the enlarged entry/exit holes and the presence of frass near the opening ( $N = 22$  larvae collected). A number of larvae drawn from the larger sample collected ( $N = 5$ ) were each housed with a captive colony of *C. mimosae* for observation of the interactions between the ants and the caterpillar. Queenright colonies of *C. mimosae* were maintained in plastic nest boxes lined with Fluon to prevent ant escape. Workers were provided with access to water from glass test-tubes half-filled with water with tight cotton plugs that allowed slow seepage of water to the workers. Intact domatia were left in the corner of the nest boxes, and workers were fed honey water and dead insects.

To test for female *A. usamba* host plant choice and the ability to discriminate between ants, a simple experiment was carried out inside a  $4 \times 4$ -m<sup>2</sup> netted field house at Kitengela (Oloorsirkon). Gravid females were collected in the field directly after mating and kept individually in a separate field house. Four potted saplings of *A. drepanolobium*, each inhabited by one of the three major ant species, *C. mimosae*, *C. nigriceps* and *T. penzigi*, and one with no ants, were placed in an arena in the field house. Females ( $N = 31$ ) were then released into the area individually and their behaviour was recorded until they laid eggs or perched in a corner of the field house. Saplings were exchanged between trials and eggs/foilage were removed when females oviposited. A total of 124 saplings was used during the course of the experiment. No sapling was used more than once. Saplings with ants were collected from areas of savannah in which no butterflies were observed, potted, sprayed down with water and held in a

netted shade house until use in the trials. Saplings without ants were grown from seed and held in a separate netted shade house until use in the trials. Saplings were misted with water using a hand-held sprayer before the trials within the netted house to create a more conducive environment for oviposition. Saplings were placed in the field house, 2 m apart (this was the distance between the closest points of any branches) and about 1 m from the sides of the field house. The saplings were arranged in a random pattern for each trial. The experiment was conducted opportunistically when females were available between May and September 2007. Females were followed for one bout of egg-laying during a 4-h time window from c. 09.00 to 13.00 h, when they typically retreated to a corner of the field house to perch. Additional observations of female oviposition behaviour were made in nature at all sites at which ants had already been identified from previous fieldwork. When a female *A. usamba* was seen ovipositing, the presence and type of ant were recorded. Females were followed in the field and watched closely to see where they were ovipositing. The species of ant on the trees on which they oviposited was recorded. Statistical analyses of these patterns were performed in Excel and R.

In order to investigate whether *A. usamba* feed on ant brood or via trophallaxis (being fed regurgitated liquid food) within the domatia, we used stable isotope analysis to match butterflies, ants and plant material, all collected from specific trees on which female *A. usamba* were observed to be ovipositing. Samples of matched ant–plant–lycaenids were collected and dried in vials with silica gel crystals. This was performed at both Suyian (Laikipia District) and Kitengela (Oloorsirkon, Kajiado District). This method is a useful tool for matching ants and lycaenids, where rearing and observations of life histories are unknown or difficult to determine, and has been widely used in studies of ant trophic levels (e.g. Davidson *et al.*, 2003). Obligate scale insects (*Stictococcus* sp.) that live in association with *C. mimosae* (Hocking, 1970) were collected and used as a reference point of a typical phytophagous insect for comparison in the stable isotope analysis. These *Stictococcus* sp. were first described as associated with *A. drepanolobium* by Richard (1976). Samples were dried overnight ( $\sim 70$  °C) and processed using standard methods for stable isotope analyses (Tieszen & Boutton, 1988; Lajtha & Michener, 1994; Webb, 1997), which included weighing, matching and packing samples in tin capsules within a standard plate, which was then shipped to the stable isotope laboratory for analysis. Analysis of both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was performed using the mass elemental analyser at the University of New Hampshire.



**Figure 1.** A, Female *Anthene usamba* ovipositing on *Acacia drepanolobium* with *Crematogaster mimosae* ants. B, Domatium of *Acacia drepanolobium* cut open to show *A. usamba* larva, attending *C. mimosae* ants and their brood. C, Freshly laid *A. usamba* egg on young foliage of the host plant. D, Tentacle organs (indicated by white arrows) everted in an *A. usamba* larva. Scale bars are 5 mm.

## RESULTS

Adult butterflies were observed on the wing at variable times of the year, but were present over most months across the years of the study (Table 1). A total of 22 larvae was recovered from domatia, pri-

marily at Kitengela (Oloorsirkon) and Ngong. All *A. usamba* larvae were found to be associating exclusively with *C. mimosae*. For the five larvae that were closely observed in the laboratory, each one associated with a different colony of *C. mimosae*; workers tending the larvae were observed spending

**Table 1.** Months in which adult *Anthene usamba* were observed on *Acacia drepanolobium* in Kenya at the two study sites

Year	J	F	M	A	M	J	J	A	S	O	N	D
Kajiado North												
2005					•			•				•
2006				•	•				•			•
2007					•	•			•			•
2008	•				•	•						
2009						•						
2010	•	•			•	•	•				•	•
2011					•		•	•		•	•	
Laikipia												
2005						•		•	•			•
2006			•	•	•				•			•
2007	•				•	•			•			•
2008	•				•	•						
2009						•						
2010	•				•	•	•				•	•
2011	•				•	•	•	•		•	•	

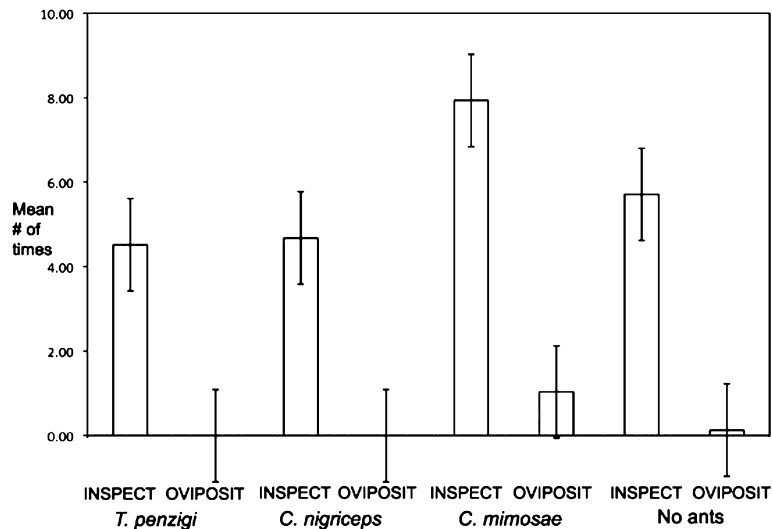
extended periods standing on the larvae and feeding from the dorsal nectary organ (mean time spent at the dorsal nectary organ,  $19.7 \pm 7.7$  s,  $N = 5$ ) Typically one, and occasionally two, ants were observed at the dorsal nectary organ (mean number of ants tending,  $1.5 \pm 0.74$ ). Close observations made on the five larvae revealed that they possess tentacle organs (TOs), and eversion of these seemed to be associated with extended ant tending (mean time spent tending after eversion of TOs,  $147.6 \pm 44.3$  s; mean number of ants tending,  $3.6 \pm 1.34$ , Fig. 1). Of the 22 larvae collected, 15 were reared to the pupal stage and, of these, ten to imago. All of the reared individuals were late-instar larvae that seemed to require little feeding. Pupation occurred inside the domatium of the acacia, with the pupa attached to the wall of the domatium. Following eclosion, the teneral (freshly emerged from the pupal stage with wings still not fully hardened) adult struggled out of the opening and then rested on the outside of the domatium or nearby foliage until its wings were fully expanded and hardened. All males were seen to disperse widely, whereas females remained in the vicinity of their natal tree. Both males and females were seen nectaring from the flowers of the host acacia (when available), as well as from adjacent flowers (*Acacia seyal*, *Plectranthus* sp., *Ocimum* sp., *Gutenbergia* sp.).

#### RESULTS OF OVIPOSITION EXPERIMENT

In the field house experiment, female *A. usamba* almost always first oviposited on saplings with *C. mimosae*: 29 of the 30 recorded first oviposition events

were on saplings with *C. mimosae*, which differs significantly from the expected counts if females are equally likely to oviposit on any of the saplings ( $\chi^2 = 82.3$ ,  $P < 0.001$ ; Fig. 2); the single remaining female failed to oviposit at all within 4 h. Following the first oviposition event, females occasionally oviposited on saplings with no ants. The single first oviposition event that was not on a *C. mimosae*-occupied tree was on a plant with no ants; a further three females were observed ovipositing on saplings without ants, after first ovipositing on a tree with *C. mimosae*, but data were not systematically collected after the first oviposition event. Females never first oviposited on saplings occupied by *T. penzigi* or *C. nigriceps*, and were never seen to do so after the first oviposition.

All females were observed to flutter about the saplings prior to the first oviposition, perhaps 'inspecting' the species of ant present. However, the degree of inspection varied significantly between the ant treatments. A mixed effects model with ant occupant as a fixed factor and female as a random factor performed significantly better than the nested random effects model without ant occupant as a predictor (Likelihood Ratio test on Maximum Likelihood models:  $\ln(L_{\text{full}}) = -302.1$ ,  $\ln(L_{\text{reduced}}) = -315.6$ ,  $P < 0.001$ ). The same mixed effects model also failed to perform significantly better than the nested fixed effects model, thus providing little evidence for variation among the propensity of females to inspect the saplings (Likelihood Ratio test on Restricted Maximum Likelihood models:  $\ln(L_{\text{full}}) = -301.2$ ,  $\ln(L_{\text{reduced}}) = -301.2$ ,  $P = 0.27$ ). For females followed in nature ( $N = 40$ ), all were seen to oviposit



**Figure 2.** Results of oviposition experiment with *Anthene usamba* showing that female butterflies oviposit preferentially on trees with *Crematogaster mimosae* ants.

exclusively on trees with *C. mimosae*, despite the close proximity and availability of trees with other ants in the habitat. All eggs were laid singly on young foliage or at the base of new growth on the upper branches of the acacia. Typically, one egg was laid per branch, with females resting or moving between trees in between oviposition events.

#### STABLE ISOTOPE ANALYSIS RESULTS

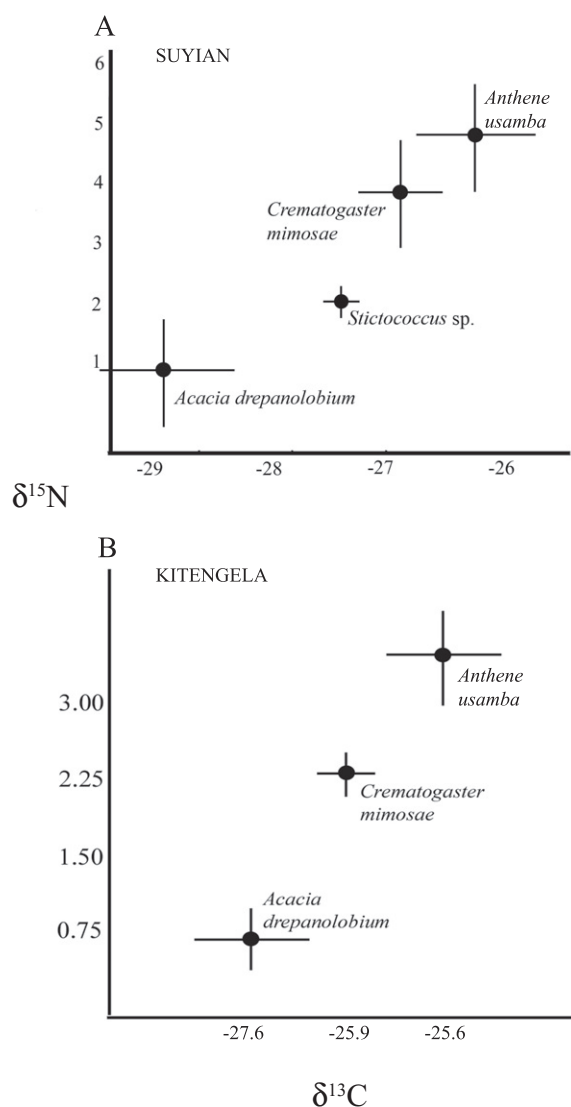
Results of the stable isotope analysis indicated that *A. usamba* is enriched in  $\delta^{15}\text{N}$  relative to its host plant, *A. drepanolobium*, and to herbivorous scale insects feeding on *A. drepanolobium* and to the host ant *C. mimosae* (Fig. 3). The level of enrichment is within that expected for a step change in trophic level between the host ant and the butterfly (Tieszen & Boutton, 1988; Lajtha & Michener, 1994; Webb, 1997). These results support the hypothesis that the larvae are aphytophagous and quite likely to consume ant brood as a source of food, although it is possible that the adult ants also feed the larvae via trophallaxis inside the domatia, where such behaviour would be difficult to observe. Similar trophic results were obtained from both study sites containing geographically separate populations of *Anthene*. The stable isotope data match observations made on the behaviour of the butterflies: they are strictly associated with *C. mimosae*, and caterpillars have only been found inside domatia. The results for the *Stictococcus* scale insects indicate where a predicted obligate herbivore should fall relative to  $\delta^{15}\text{N}$ . We also found that *A. usamba* are slightly enriched in  $\delta^{13}\text{C}$  relative to the acacia and ants; this can be explained by the different

rates of fractionation that are expected as part of natural variation in the muscle tissue to chitin ratios in ant bodies versus butterfly wings (e.g. Lajtha & Michener, 1994). Therefore, the  $\delta^{13}\text{C}$  axis indicates that these species are intimately connected with one another, providing further support to the observations of an obligate ant–lycaenid association.

#### DISCUSSION

The lycaenid butterfly, *A. usamba*, appears to be an obligate myrmecophile of the acacia ant, *C. mimosae*. The host tree, *A. drepanolobium*, is the only host in which *C. mimosae* are known to occur as obligate plant ants. Our results suggest that *A. usamba* is a parasite of the mutualism between the ant and the acacia. This needs to be tested further with more detailed studies of the exact nature of the interaction between the larvae and the ants. As this is the first documented example of an obligate ant association and aphytophagy in African *Anthene*, this study indicates that other African lycaenid species associated with *A. drepanolobium* should be evaluated for ant association and aphytophagy.

Not all lycaenid pupae are tended by ants, but highly myrmecophilous species, such as *Jalmenus evagoras* in Australia, are well known to be associated with ants throughout the pupal period (Pierce & Nash, 1999), and the pupae of many lycaenid species use stridulations to communicate with associated ants (Downey & Allyn, 1978; Pierce *et al.*, 2002). The location of both larvae and pupae of *A. usamba*, as well as observations of adult



**Figure 3.** Stable isotope data showing aphytophagy of *Anthene usamba*. A, Data from Suyian, Laikipia District. Note *Anthene usamba* is higher in the trophic scale than the host ant, *Crematogaster mimosae*, and the obligately herbivorous scale insect, *Stictococcus* sp. B, Stable isotope data from Kitengela (Oloorsirkon), Kajiado North District. Results from this site support the observations from Suyian, Laikipia District.

butterflies around *A. drepanolobium* trees, is further supported by the stable isotope data which show that the species are linked. The stable isotope results indicate that this is a useful tool for understanding trophic interactions between insects, plants, predators and parasites on African acacias (Martins, 2013). The use of stable isotopes in this study enabled the assessment of the trophic interactions at a population level with a quantitative

measure ( $\delta^{15}\text{N}$ ). In addition, we believe that  $\delta^{13}\text{C}$  can also be a useful measure when it shows little enrichment or variation, as in this study. This lack of enrichment indicates the close association of partners (i.e. obligate symbioses). If variation is observed in the  $\delta^{13}\text{C}$  axis, this can be a useful measure of how much of a diet of a given species is derived from the host acacias (C3 plants) versus the surrounding grasslands (C4 plants). For example, if a lycaenid feeds on ant brood or is fed by ants that are also deriving a significant portion of their diet from nonacacia sources (such as grass-dwelling insect herbivores), more variation in  $\delta^{13}\text{C}$  would be predicted. This pattern of C3 trees in savannahs of C4-dominated grasses (Cerling, Levin & Passey, 2011) should be taken into consideration in future studies of insect–plant interactions on the African savannah, and has the potential to aid in the dissection of the diets of many extant savannah insect species.

The interaction between *A. usamba* and ants appears, at first, to be a specialized mutualism in which butterflies associate exclusively with colonies of *C. mimosae*, and both partners derive benefits. However, given our results from the stable isotope analysis, the larvae are likely to be feeding on ant regurgitations and/or ant brood as well as host plant foliage, and whether this is a facultative or obligate relationship remains to be investigated more closely. One of us (DJM) has observed that the adults are often abundant at widely different times of year (Table 1), even when the rains fail (drought years of 2008–2009) and other phytophagous lycaenid species are absent. At that time, most of the larvae recovered were from domatia that also contained ant brood. Aphytophagy is well known from other lycaenids (Cottrell, 1984; Pierce, 1995), and our results show that *A. usamba* is aphytophagous and exploits the *C. mimosae* ants for food, through trophallaxis, myrmecophagy or both. Here, the application of stable isotopes as a method has been shown to have great potential for the dissection of the life histories of other ant–plant–lycaenid interactions in which larvae are difficult to observe because they spend time either in the domatia or the ant nest, and the exact nature of the interaction remains unclear. The clear nitrogen enrichment of the adult *Anthene* demonstrates that stable isotopes can reveal information on the specificity of life history strategies in settings such as these, where larvae are hidden and/or hard to locate in numbers. Given the number of different lycaenid species that occur in association with acacias and ants in eastern Africa (Table 2), there is the potential to expand these methods to understand lycaenid life history strategies at the landscape level. For example, in the diverse African genus

**Table 2.** Records of lycaenids directly observed associating with ant-acacias in Kenya (K) and Tanzania (TZ) during this study. Eighteen lycaenid species were found on ant-acacias occupied by ants. Comparisons are made for each species with what was known about the life history of each species in the available literature. Our observations suggest that an even greater number of lycaenids are potentially closely associated with acacia ants, and the full nature of these interactions remains to be documented. Note: all sites from Kenya, with sites from Tanzania indicated in parentheses. All host plant/ant records are taken from Jackson (1937), Boulard (1968), Kielland (1990) and Larsen (1991). All host plants were previously recorded as 'Acacia'

Lycaenid butterfly species observed on ant-acacias during this study	Ant association if known (literature – note citations are the same for host plants)	Ant observations made during this study	Host plant observations made during this study	Localities in which observed
<i>Lachnoctema</i> sp.*	Unknown	<i>C. mimosae</i>	<i>Acacia drepanolobium</i>	Laikipia (K)
<i>Paraphnaeus hutchinsoni</i>	Unknown	<i>C. mimosae</i>	<i>A. drepanolobium</i>	Laikipia (K)
<i>Spindasis nyassae</i>	Unknown	<i>Crematogaster</i> sp.	<i>A. seyal</i>	Lake Victoria (K)
<i>Spindasis ella</i>	<i>Pheidole</i> (?)		<i>A. drepanolobium</i>	Kitengela (K)
<i>Spindasis tavetensis</i>	<i>Pheidole</i> (?)	<i>Crematogaster</i> sp.	<i>A. zanzibarica</i>	Kasigau (K)
<i>Chloroselas minima</i>	Unknown	<i>C. mimosae</i>	<i>A. drepanolobium</i>	Laikipia (K)
<i>Axiocerces harpax ugandana</i>	<i>Pheidole</i>	<i>Crematogaster</i> sp.	<i>A. drepanolobium</i>	Ngong Hills (K)
<i>Axiocerces amanga</i>	<i>Crematogaster</i> (?)			
<i>Deudorix dinochares</i>	<i>Camponotus</i> (?)		<i>A. drepanolobium</i>	Laikipia (K)
<i>Deudorix suk</i>	Unknown	<i>Crematogaster</i> sp.	<i>A. drepanolobium</i>	Kajiado (K)
<i>Anthene definita</i>	<i>Pheidole</i> (?)	<i>Crematogaster</i> sp.	<i>A. seyal</i>	Kerio Valley (K)
<i>Anthene otacilia</i>	'Tended by ants'	<i>Crematogaster</i> sp.	<i>A. drepanolobium</i>	Laikipia, Kitengela (K)
<i>Anthene usamba</i>	<i>Crematogaster</i>	<i>C. nigriceps</i>	<i>A. drepanolobium</i>	Laikipia Kitengela (K)
	<i>Pheidole</i> (?)	<i>C. mimosae</i>	<i>A. drepanolobium</i>	Laikipia Kitengela (K)
<i>Anthene amarah</i>	'Tended by ants'	<i>Crematogaster</i> sp.	<i>A. zanzibarica</i>	Pangani (TZ)
<i>Triclema nigeriae</i>	'Tended by ants'	<i>Crematogaster</i> sp.	<i>A. drepanolobium</i>	Kajiado (K)
<i>Eicochrysops nandianus</i>	Unknown	<i>Crematogaster</i> sp.	<i>A. drepanolobium</i>	Kerio Valley (K)
<i>Chilades kedonga</i>	Unknown	<i>C. nigriceps</i>	<i>A. drepanolobium</i>	Kajiado (K)
		<i>C. mimosae</i>	<i>A. drepanolobium</i>	Laikipia
<i>Virachola ecauta</i>		<i>Crematogaster</i> sp.	<i>A. drepanolobium</i>	Kajiado
			<i>A. pseudofistula</i>	Ngong (K)
				Ruaha (TZ)

\*This lycaenid species is aphytophagous and most likely feeding on scale insects on *A. drepanolobium*.



*Lepidochrysops*, which has proved difficult to study as these butterflies exploit subterranean ant nests, stable isotopes may also help to solve life history strategy questions.

The presence of this lycaenid on the ant-acacia *A. drepanolobium*, as an obligate myrmecophile with the ant *C. mimosae*, as indicated by the results of the oviposition experiment, is worth noting, especially given the large areas of eastern Africa covered by these acacias (Beentje, 1994). Further work on the biogeography of these associations would be worth undertaking, given their wide distributions, and the interaction we document supports the idea of specialized interactions being more common among insects (Loxdale *et al.*, 2011). Ant-dependent oviposition has been demonstrated for other mutualistic lycaenid species, including *Jalmenus evagoras* (Pierce & Elgar, 1985) and *Hemiargus isola* (Wagner & Kurina, 1997), but this is not the case for all lycaenids. For example, experiments with *Maculinea* species that parasitize the nests of *Myrmica* ants have failed to show ant-dependent oviposition (e.g. Furst & Nash, 2010). The life histories of many East African lycaenids, especially in seasonal savannahs and drylands, remain largely unknown (Larsen, 1991). In addition, recent work rearing Lepidoptera (Tortricidae and Pyralidae) from ant-acacias has yielded interesting new records and species (Agassiz, 2011). The data on ant-dependent oviposition indicate that ant-associated acacias may be an important component of host plant–host ant diversity, contributing to the patterns observed not only in lycaenids (Table 2), but also in other myrmecophilic groups.

The observations of lycaenids on acacias compared with records from the literature indicate that a number of lycaenids are associated with acacias and their ants in Kenya, including several species of *Anthene*, *Chilades* and other Polyommatainae (Table 2). Our observations suggest that ant-acacias are an important resource for ant-associated lycaenids. Many of these lycaenids are rare or localized, and could be of interest for conservation monitoring, as in the case of phytoreplicative species from the Palaearctic genus *Maculinea* (Thomas, 1980, 1983). The relationship uncovered in this study hints at many more fascinating interactions that remain to be explored in the ant–acacia system.

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#### REFERENCES

- Agassiz DJL.** 2011. The Lepidoptera of *Acacia domatia* in Kenya with description of two new genera and six new species. *Journal of Natural History* **45**: 1867–1893.
- Atsatt PR.** 1981a. Ant-dependent food plant selection of the mistletoe butterfly *Ogyris amaryllis* (Lycaenidae). *Oecologia* **48**: 60–63.
- Atsatt PR.** 1981b. Lycaenid butterflies and ants: selection for enemy-free space. *American Naturalist* **118**: 638–654.
- Beentje H.** 1994. *Kenya trees, shrubs and lianas*. Nairobi: National Museums of Kenya.
- Bennun L, Njoroge P.** 1999. *Important bird areas in Kenya*. Nairobi: The East Africa Natural History Society.
- Boulard M.** 1968. Documents sur deux Lepidopteres Lycaenidae predateurs d'Homopteres. *Cahiers de Maboko (Paris)* **6**: 117–126.
- Braby M.** 2000. *Butterflies of Australia: their identification, biology and distribution*. Melbourne: CSIRO Publishing.
- Cerling TE, Levin NE, Passey BH.** 2011. Stable isotope ecology in the Omo-Turkana Basin. *Evolutionary Anthropology* **20**: 228–237.
- Clark GC, Dickson CGC.** 1971. *Life histories of the South African lycaenid butterflies*. Cape Town: Purnell.
- Common IFB, Waterhouse DF.** 1981. *Butterflies of Australia*. Sydney: Angus & Robertson.
- Cottrell CB.** 1984. Aphytophagy in butterflies: its relationship to myrmecophily. *Zoological Journal of the Linnean Society* **79**: 1–57.
- Davidson DW, Cook SC, Snelling RR, Chua TH.** 2003.

- Explaining the abundance of ants in lowland tropical rainforest canopies. *Science* **300**: 969–972.
- Downey JC, Allyn AC. 1978.** Sound produced in pupae of Lycaenidae. *Bulletin of the Allyn Museum* **48**: 1–13.
- Farquharson CO. 1921.** Five years' observations (1914–1918) on the bionomies of southern Nigerian insects, chiefly directed to the investigation of lycaenid life-histories and to the relation of Lycaenidae, Diptera and other insects to ants. *Transactions of the Royal Entomological Society of London* **73**: 31–448.
- Fiedler K, Maschwitz U. 1988.** Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and lycaenids (Lepidoptera: Lycaenidae). 111. New aspects of the function of the retractile tentacular organs of lycaenid larvae. *Zoologische Beiträge (Neue Folge)* **31**: 409–416.
- Fiedler K, Maschwitz U. 1989.** The symbiosis between the weaver ant, *Oecophylla smaragdina* and *Anthene emolus*, an obligate myrmecophilous lycaenid butterfly. *Journal of Natural History* **23**: 833–846.
- Furst MA, Nash DR. 2010.** Host ant independent oviposition in the parasitic butterfly, *Maculinea alcon*. *Biology Letters* **6**: 174–176.
- Heath A, McLeod L, Kaliszewska ZA, Fisher CWS, Cornwall M. 2008.** Field notes including a summary of trophic and ant-associations for the butterfly genera *Chrysoritis* Butler, *Aloeides* Hübner and *Thestor* Hübner (Lepidoptera: Lycaenidae) from South Africa. *Metamorphosis* **19**: 127–148.
- Henning SF. 1983.** Chemical communication between lycaenid larvae (Lepidoptera: Lycaenidae) and ants (Hymenoptera: Formicidae). *Journal of the Entomological Society of Southern Africa* **46**: 341–366.
- Hinton HE. 1951.** Myrmecophilous Lycaenidae and other Lepidoptera – a summary. *Proceedings and Transactions of the Southern London Entomological and Natural History Society 1949–50* 111–175.
- Hocking B. 1970.** Insect associations with swollen thorn acacias. *Transactions of the Royal Entomological Society of London* **122**: 211–255.
- Jackson THE. 1937.** The early stages of some African Lycaenidae (Lepidoptera) with an account of their larval habits. *Transactions of the Royal Entomological Society, London* **86**: 201–238.
- Jackson THE. 1947.** The early stages of some African Lycaenidae. *Proceedings of the Royal Entomological Society, London* **A22**: 44–48.
- Kielland J. 1990.** *The butterflies of Tanzania*. Melbourne and London: Hill House Publishers.
- Kuria SK. 2006.** The role of ants in structuring insect communities on the canopies of *Senegalia drepanolobium* near Laikipia, Kenya. PhD Thesis, Rhodes University.
- Lajtha K, Michener RH. 1994.** *Stable isotopes in ecology and environmental science*. Oxford: Blackwell Scientific Publications.
- Lamborn WA. 1913.** On the relationship between certain West African insects, especially ants, Lycaenidae and Homoptera. *Transactions of the Royal Entomological Society, London* **65**: 436–498.
- Larsen TB. 1991.** *The butterflies of Kenya and their natural history*. Oxford: Oxford University Press.
- Libert M. 2010.** *Revision des Anthene Africains*. Nairobi: Lambillionea and African Butterfly Research Institute.
- Loxdale HD, Lushai G, Harvey JA. 2011.** The evolutionary improbability of 'generalism' in nature, with special reference to insects. *Biological Journal of the Linnean Society* **103**: 1–18.
- Martins DJ. 2010.** Not all ants are equal: obligate acacia ants provide different levels of protection against mega-herbivores. *African Journal of Ecology* **48**: 1115–1122.
- Martins DJ. 2013.** Effect of parasitoids, seed-predators and ant-mutualists on fruiting success and germination of *Acacia drepanolobium* in Kenya. *African Journal of Ecology*. doi: 10.1111/aje.12067
- Moore G, Smith GF, Figueiredo E, Demissew S, Lewis G, Schrire B, Rico L, van Wyk AE. 2010.** *Acacia*, the 2011 Nomenclature Section in Melbourne, and beyond. *Taxon* **59**: 1188–1195.
- Palmer TM, Doak DF, Stanton ML, Bronstein JL, Kiers ET, Young TP, Goheen JR, Pringle RM. 2011.** Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 17 234–17 239.
- Palmer TM, Stanton ML, Young TP. 2002.** Burning bridges: priority effects and the persistence of a competitively subordinate acacia-ant in Laikipia, Kenya. *Oecologia* **133**: 372–379.
- Palmer TM, Young TP, Stanton ML. 2000.** Short-term dynamics of an acacia ant community. *Oecologia* **123**: 425–435.
- Pierce NE. 1983.** The ecology and evolution of symbioses between lycaenid butterflies and ants. PhD Dissertation, Harvard University.
- Pierce NE. 1985.** Lycaenid butterflies and ants: selection for nitrogen-fixing and other protein-rich food plants. *American Naturalist* **125**: 888–895.
- Pierce NE. 1995.** Predatory and parasitic Lepidoptera: carnivores living on plants. *Journal of the Lepidopterists' Society* **49**: 412–453.
- Pierce NE, Braby MF, Heath A, Lohman DJ, Mathew J, Rand DB, Travassos MA. 2002.** The ecology and evolution of ant-association in the Lycaenidae (Lepidoptera). *Annual Reviews of Entomology* **47**: 733–771.
- Pierce NE, Eastal S. 1986.** The selective advantage of attendant ants for the larvae of a lycaenid butterfly *Glaucopsyche lygdamus*. *Journal of Animal Ecology* **55**: 451–462.
- Pierce NE, Elgar MA. 1985.** The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid butterfly. *Behavioural Ecology and Sociobiology* **16**: 209–222.
- Pierce NE, Kitching RL, Buckley RC, Taylor MFJ, Benbow KF. 1987.** The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants. *Behavioural Ecology and Sociobiology* **21**: 237–248.

- Pierce NE, Mead PS. 1981.** Parasitoids as selective agents in the symbiosis between lycaenid butterfly larvae and ants. *Science* **112**: 1185–1187.
- Pierce NE, Nash DR. 1999.** The Imperial Blue, *Jalmenus evagoras* (Lycaenidae). In: Kitching R, Sheermeyer E, Jones R, Pierce NE, eds. *The biology of Australian butterflies (monographs on Australian Lepidoptera Vol. 6)*. Sydney: CSIRO Press, 277–316.
- Richard C. 1976.** Révision du groupe des *Stictococcus*, et création de taxa nouveaux (Homoptera, Coccoidea) (Revision of the *Stictococcus* and description of new taxa (Homoptera Coccoidea). *Annales de la Société Entomologique de France* **12**: 653–669.
- Saarinen EV. 2006.** Differences in worker caste behaviour of *Oecophylla smaragdina* (Hymenoptera: Formicidae) in response to larvae of *Anthene emolus* (Lepidoptera: Lycaenidae). *Biological Journal of the Linnean Society* **88**: 391–395.
- Shorrocks B. 2007.** *The biology of African savannahs*. Oxford: Oxford University Press.
- Stanton ML, Palmer TM, Young TP, Evans A, Turner ML. 1999.** Sterilization and canopy modification of a swollen thorn acacia by a plant-ant. *Nature* **401**: 578–580.
- Stanton ML, Palmer TP. 2011.** The high cost of mutualism: effects of four species of East African ant symbionts on their myrmecophyte host tree. *Ecology* **92**: 1073–1082.
- Thomas CD. 1985.** Specializations and polyphagy of *Plebejus argus* (Lepidoptera: Lycaenidae) in North Wales. *Ecological Entomology* **10**: 325–340.
- Thomas JA. 1980.** The extinction of the Large Blue and the conservation of the Black Hairstreak butterflies (a contrast of failure and success). *Annual Reports of the Institute of Terrestrial Ecology* **1979**: 19–23.
- Thomas JA. 1983.** The ecology and conservation of *Lysandra bellargus* (Lepidoptera: Lycaenidae) in Britain. *Journal of Applied Ecology* **20**: 59–83.
- Tieszen IL, Boutton TW. 1988.** Stable carbon isotopes in terrestrial ecosystem research. In: Rudel RW, Ehlinger JR, Nagy KA, eds. *Stable isotopes in ecological research: ecological studies, Vol. 68*. Berlin, New York, Heidelberg: Springer, 117–195.
- Wagner D, Kurina LM. 1997.** The influence of ants and water availability on oviposition behaviour and survivorship of a facultatively ant-tended herbivore. *Ecological Entomology* **22**: 352–360.
- Webb SC. 1997.** Carbon and nitrogen stable isotopes: the influence of diet. Doctoral Thesis, Oxford University.
- Woodhall S. 2005.** *Field guide to butterflies of South Africa*. Cape Town: Struik Publishing.
- Young TP, Stubblefield CH, Isbell L. 1997.** Ants on swollen-thorn acacias: species coexistence in a simple system. *Oecologia* **109**: 98–107.