Five blues on a flower: interactions between Polyommatinae butterflies (Lepidoptera, Lycaenidae), ants and parasitoids in the northern Peloponnese (Greece)

Tristan Lafranchis and Antoine Lafranchis
Tristan and Antoine Lafranchis, GR-25003 Dhiakopto, Greece; lafranch@otenet.gr

Abstract: A field study undertaken on the slopes of Mt. Klokos (Peloponnese, Greece) completed by rearings in 2005–2007 has revealed the particular relationship between the community of Polyommatinae caterpillars feeding on the sainfoin Onobrychis ebenoides Boiss. & Spruner, their parasitoids and 10 species of attending ants.

Methodology
The study area lies on the north-facing slope of Mt. Klokos (northern Peloponnese) between 1100 and 1200 m. It has been chosen for its large populations of Polyommatinae and for its easy access. We could therefore hope to find caterpillars easily and visit it regularly. The substrate is limestone, with a stony and rocky soil. A track runs along the edge of a dense woodland of Greek Fir (Abies cephalonica Loudon, Pinaceae) and through dry grasslands and open scrub. The open habitats are locally dominated by patches of Onobrychis ebenoides Boiss. & Spruner, Vicia tenuifolia Roth or Anthyllis vulneraria L., all Fabaceae which host the larvae of several species of Polyommatinae. The study was undertaken in spring 2005, 2006 and 2007 focusing on the species using the sainfoin Onobrychis ebenoides as a larval foodplant.

In 2005, we collected 14 caterpillars of Agrodiaetus which were reared individually in small plastic film boxes and fed with cut leaves. The boxes were checked every day. Most larvae and pupae were photographed and butterflies were released after identification. We could find the 3 species of Agrodiaetus flying on the site — riparti (Freyer, 1830) (Figs. 8–13), aroaniensis Brown, 1976 (Figs. 3–7) and admetus (Esper, [1783]) (Figs. 1–2) — and guessed that identification was possible at the larval stage thanks to differences in the markings on last instar larvae as found for the Italian species by Bolognesi (2000). As these markings seemed to be variable, the distinctive features had to be tested again on more specimens.

In 2006 a more extensive study was undertaken on the same site, with 7 field excursions of 4–7 hours each and the rearing of more than 50 caterpillars of Agrodiaetus in the same successful conditions as in 2005. All these caterpillars were provisionally identified using the features found during the previous season. When the butterflies had hatched, their identity was compared with the tentative identification of the larvae: on 49 predictions, only 28 were correct. The variability of the markings precludes any identification at the larval stage especially between A. riparti and A. aroaniensis. The differences are more constant (75%) between A. admetus and A. riparti, the larvae of the former often lack the lateral purple stripe (but not always, 25% of the caterpillars show this stripe, sometimes heavily marked). We also decided to monitor as many larvae as possible in the field. Each stem of the foodplant with a caterpillar was marked by a coloured thread and a simple code written on a flat stone put at the bottom of the stem.

Introduction
The relationship between the caterpillars of Lycaenidae and ants have been thoroughly studied for more than a century in various countries. Most authors have interpreted the myrmecophily of Lycaeinae as an exchange, the ants getting a rich nutriment from the caterpillars (Cottrell 1984, Cushman et al. 1994, Fiedler 1995, Fiedler & Maschwitz 1988, Fiedler & Saam 1995, Pierce 1983) which in return get protection against predators and parasitoids from the ants (Fiedler 1989, 1991, 2001, 2006, Pierce & Eastseal 1986, Pierce & Mead 1981, Pierce et al. 1987, Pierce et al. 2002, Seufert & Fiedler 1994). Nevertheless only few studies have been made at a community level (Forister et al. 2011).

Information on the biology of Greek Lycaeinae is scarce and scattered (Tolman 1993, 1995c, Tolman & Lewington 1997, Lafranchis 2004). Facultative mutualism with ants has been reported only for 19 species on the 52 Polyommatinae known to occur in Greece (Tolman 1994, 1995a, 1995b, Tolman & Lewington 1997, Lafranchis et al. 2007, Lafranchis in preparation). We have therefore tried to investigate the relationships between 5 Polyommatinae butterflies linked to the endemic sainfoin Onobrychis ebenoides Boiss. & Spruner (Fabaceae) and ants and parasitoids in the northern Peloponnese (Greece). Larval monitoring in the field has been combined with rearing to check whether identification of Agrodiaetus (after other authors Agrodiaetus is a subgenus of Polyommatus) species was possible at the larval stage and to assess the importance of myrmecophily and its possible effect on parasitism. As parasitism at larval stage was found to be very low, Agrodiaetus caterpillars collected in two other places in northern Greece were reared to get comparative information.

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This proved to be efficient as it was usually very easy to locate almost all these plants a few days or a few weeks later. On each visit, the caterpillars found were measured and the number of attending ants was noted, as well as their genus for the most distinctive ones. This was also done for the caterpillars of two other species of blues sharing the same foodplant, *Glaucopsyche alexis* (Poda, 1761) (Figs. 15–19) and *Polyommatus thesrites* (Cantuér, [1835]) (Fig. 14). 258 caterpillars of the 5 species were monitored in the field.

In 2007 the site was visited 4 times and 56 caterpillars were collected to be reared. 195 other caterpillars were left in the field once identified (at generic level only for the *Agrodiaetus*), measured and their attending ants noted or captured.

We had planned to go on monitoring the populations of blues on that site in the following years, but the northern side of the mountain from 100–1500 m was completely burnt in July 2007 during one of the disastrous fires which destroyed several areas in the Peloponnesse. Though most butterfly populations were not strongly affected, the number of *Onobrychis ebenoides* was much lower in spring 2008 but had quickly recovered to its former density by 2010.

**Life-cycles**

The only plurivoltine blue studied is *Polyommatus thesrites* which produces 3 broods a year here: from late April to late May, late June to July and September. Females lay eggs on *Onobrychis* (on Mt. Klokos mostly on *O. ebenoides*, sometimes on *O. aequidentata* Sml.), on the flower-buds or on the leaflets. The pupal stage lasts 17–24 days. Hibernation is initiated by 3rd instar caterpillars which complete their growth in April.

*Glaucopsyche alexis* lay eggs on the calyx of the flower-buds in late April–May and they hatch after 7–9 days. Caterpillars spend their life on the flower spike, feeding on buds and flowers. They complete their growth in 3–4 weeks, then they bury themselves just below the ground. Caterpillars are variable and mostly dimorphic on the studied area: 78% are pink and 20% are green. A single deep yellow caterpillar has been found and 2 grey ones were also noted.

The three *Agrodiaetus* species have a similar biology. Butterflies usually start to hatch on Mt. Klokos around mid-June. They emerge between 6 and 8 o’clock in the morning. The flight season is long, averaging 3 months for the 3 species but we have not tried to survey the imaginal populations and have no information on their life-span. The sex-ratio of reared specimens is close to 1. Males look for females patrolling the breeding areas in a low flight. We have not observed the courtship but we guess the pheromones released by the andoconias play an important role in the recognition of mates, as visual stimuli could be problematic in a place where 3 similar-looking *Agrodiaetus* species fly together. Female butter-

flies lay eggs from July to mid-September on the dry flower-stems of *Onobrychis ebenoides*, usually in the axil of a bract, either when the fruit is still attached or when it has already fallen to the ground. They hatch in September or October in the wild (all the egg-cases checked in November were empty). The very young caterpillars hibernate. Only *A. admetus* has been found feeding on another foodplant, the annual *Onobrychis aequidentata*.

The smallest *Agrodiaetus* caterpillar we could find in the wild was a 3rd instar larva about 4 mm long found on 1. v. 2005 in northern Greece. Feeding on the underside of a leaf, its presence was revealed by 2 small ants attending it. When we started to monitor the larvae on Mt. Klokos on 10. v. 2006 most were already in the 4th and 5th instar. On 14. v., we had 2 L4 (less than 5 mm long), 23 L3 (5–8 mm) and 30 L2 (more than 8 mm). On 24. v. on 51 measured caterpillars there were 3 L5 and 48 L4. There were certainly also pupae as several larvae which had already reached the maximum size (13–17 mm) on 14. v. were not seen again later. On 30. v. all the *Agrodiaetus* caterpillars were at the end of the last instar and their numbers were decreasing. They were very scarce on 2. vi. with only 3 L5 found. As for many Polyommatinae, the caterpillar pupates just beneath the ground.

Fresh pupae of the 3 *Agrodiaetus* are green. They become yellowish 1–2 weeks later and turn dark brown a few days before the butterfly hatches. The pupal stage lasts about 3 weeks in captivity and it is certainly also the case in the wild as the first *Agrodiaetus* hatched on Mt. Klokos at the same time as at home (3rd week of June in 2006 and 2007). There seems to be some difference in the length of the pupal stage, especially between *A. ripartii* and *A. aroaniensis*. The following results cumulate 2005, 2006 and 2007 rearings from the northern Peloponnesse, all the pupae kept under the same conditions (Table 1).

**Table 1:** Pupal development of *Agrodiaetus* species.

<table>
<thead>
<tr>
<th>Species</th>
<th>n pupae</th>
<th>duration min.–max. [days]</th>
<th>average [days]</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. ripartii</em></td>
<td>33</td>
<td>16–23</td>
<td>20</td>
</tr>
<tr>
<td><em>A. aroaniensis</em></td>
<td>12</td>
<td>21–25</td>
<td>23</td>
</tr>
<tr>
<td><em>A. admetus</em></td>
<td>6</td>
<td>19–24</td>
<td>21</td>
</tr>
</tbody>
</table>

We have not looked for pupae in the field.

Another polyommatine species shares the same habitat and foodplant: *Cupido osiris* (Meigen, [1829]), whose population seems rather weak, with no caterpillar found during our researches. Females lay the egg on the calyx of the flower-buds in May. The larvae, which are very mimetic with the flowers and small, complete their growth quickly. They enter diapause early June and remain completely inactive until next spring when they pupate without further feeding (observations partly in the field and in rearing from a colony close to Mt. Klokos).

The foodplant *Onobrychis ebenoides* is a perennial of dry grasslands and clearings, growing between 200 and
2000 m (1100–1250 m on Mt. Klokos) and endemic to Greece (Lafranchis & Sfikas 2009). On Mt. Klokos, flowers bloom in May, the peak being in the second half of the month. The flowering stems and the leaves dry during the summer and the mature fruits fall to the ground when dry. Fresh leaves appear in the autumn.

**Caterpillars and ants**


Additionally, a freshly emerged male of *Polyommatus thersites* was discovered in the short grass and its exuvia, still soft, was lying on the ground at the entrance of a nest of *Tetramorium cf. hippocrate* (Agosti & Collingwood, 1987) but this small yellow ant has never been found around any caterpillar in the area. Ants belonging to this genus are mostly active at night.

The attractiveness of caterpillars to ants is very strong though variable: *Glaucopsyche alexis* is certainly the most attractive amongst the studied species, its caterpillars being almost always attended by ants, as we could also observe in several sites in southern France and northern Italy (Lafranchis & Kan 2012). Interestingly, the two unattended caterpillars were the grey ones, indicating this colour could result from some health problem which may have stopped the secretion of the honey glands and kept the ants away. *Polyommatus thersites* and the 3 *Agrodiaetus* spp. (considered here together as caterpillars cannot be identified without being reared to imaginal stage) show a similar attractiveness to ants, respectively 89 and 87% of their caterpillars being found attended by ants (average on years 2006 and 2007) (Table 3). As the caterpillars of these 5 species all have the same food – the buds and flowers of *Onobrychis ebenoides* – the stronger attractiveness to ants shown by *G. alexis* does not depend on the larval diet. Nectar production by caterpillars is known to be higher in some species which are therefore more attractive to ants but individual variability in the quantity of nectar produced within a species is also very strong. The chemical composition of the nectar secretion has specific characteristics which could also explain this difference in attractiveness (Daniels 2004).

### Table 2: Ants associated with Polyommatinae on Mt. Klokos. — ++ = frequently observed association; + = observed association; – = not observed together.

<table>
<thead>
<tr>
<th>Ant species</th>
<th>Subfamily of Formicidae</th>
<th>Lycaenidae species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tapinoma simrohi</td>
<td>Dolichoderinae</td>
<td></td>
</tr>
<tr>
<td>Camponotus aethiops</td>
<td>Formicinae</td>
<td>G. alexis +</td>
</tr>
<tr>
<td>Camponotus gestroi</td>
<td>Formicinae</td>
<td>P. thersites +</td>
</tr>
<tr>
<td>Camponotus laconicus</td>
<td>Formicinae</td>
<td>A. admetus +</td>
</tr>
<tr>
<td>Camponotus oertzeni</td>
<td>Formicinae</td>
<td>A. ripartii +</td>
</tr>
<tr>
<td>Camponotus picus</td>
<td>Formicinae</td>
<td>A. aroaniensis +</td>
</tr>
<tr>
<td>Lepisiota melas</td>
<td>Formicinae</td>
<td></td>
</tr>
<tr>
<td>Plagiolepis pygmaea</td>
<td>Formicinae</td>
<td></td>
</tr>
<tr>
<td>Plagiolepis vindobonensis</td>
<td>Formicinae</td>
<td></td>
</tr>
<tr>
<td>Crematogaster sordidula</td>
<td>Myrmicinae</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Compared attractiveness of polyommatine larvae to ants in the years 2006 and 2007.

<table>
<thead>
<tr>
<th>G. alexis</th>
<th>P. thersites</th>
<th>Agrodiaetus spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N caterpillars with ants</td>
<td>50</td>
<td>85</td>
</tr>
<tr>
<td>N caterpillars total</td>
<td>51</td>
<td>86</td>
</tr>
<tr>
<td>% with ants</td>
<td>98</td>
<td>99</td>
</tr>
</tbody>
</table>

The size and the number of ants attending a caterpillar depends mostly on the size of the ants as shown in Table 4. They also depend on the size of the caterpillar (Fiedler & Hagemann 1995, Peterson 1995) but to a lesser extent as we observed large ants with small caterpillars several times. Though the smallest larvae of *G. alexis* (2–3 mm long) were found only with small ants, the smallest *Agrodiaetus* larvae found on Mt. Klokos were all attended by large *Camponotus*. On 52 larvae of *Agrodiaetus* ranging from 7–16 mm the number of attending small ants did not vary significantly with the caterpillar size. The various species of *Camponotus* are large to very large ants and no more than 5 ants have been observed together around a caterpillar, with an average of only one ant (1.39). On the contrary, the small ants, mostly *Crematogaster sordidula*, can be numerous on a caterpillar (up to 12), with an average of 4 ants (3.74) per caterpillar. Table 4 confirms the stronger attractiveness of *G. alexis* caterpillars to ants: the proportion of caterpillars attended by 2 or more *Camponotus* is definitely higher than for the other Polyommatinae. Small ants are also on average more numerous with *G. alexis* (3.8) than with *Agrodiaetus* (2.7).
Larval monitoring has shown a great fidelity in the relationship between caterpillars and ants. On 51 larvae found at least twice with ants, 44 were attended by the same species of ant (86%). This probably only reflects the territorial situation of the ant nests as there were areas where all the caterpillars of Polyommatinae were attended by *Camponotus aethiops* and other areas where the caterpillars were all with *Crematogaster sordidula*. This was easy to note along some parts of the track where the hostplant was growing in linear patches along it.

The attending ants on Mt. Klokos probably find in the many caterpillars of Polyommatinae an important source of energy during the months of May and June. In April they are often found sucking the stipules of the shoots of *Vicia tenuifolia* and the bracts of *Scorzonera laciniosa* L. They are also seen with hibernated caterpillars of *Polyommatus amandus* (Schneider, 1792) on *Vicia tenuifolia* and *P. icarus* (Rottemburg, 1775) on various small Fabaceae. During the first ten days of May, ants are very often seen walking up and down on sainfoins. They

Figs. 1–2: *Agrodiaetus admetus*, L5 larvae. Fig. 1: ♂, with 2 *Camponotus aethiops*. Mt. Smolikas (Epirus), 1200 m, 28. v. 2006. Fig. 2: ♀, on *Onobrychis ebenoides* with ants *Crematogaster sordidula*. Pteri (Peloponnese), 1200 m, 24. v. 2006. — Figs. 3–7: *Agrodiaetus araniensis*, L5 larvae on *Onobrychis ebenoides*, all Pteri, 1200 m. Fig. 3: ♀, with *Camponotus aethiops*, 11. v. 2005. Figs. 4–5: ♂, with 2 *Camponotus aethiops*, 11. v. 2005. Fig. 6: ♀, with ants *Plagiolepis vindobonensis*, 1. vii. 2005. Fig. 7: ♂, with ants *Crematogaster sordidula*, 13. v. 2005. — Figs. 8–13: *Agrodiaetus ripartii*, L5 (Figs. 8–9) and L6 (Figs. 10–13) larvae on *Onobrychis ebenoides*, most Pteri, 1200 m. Figs. 8–9: ♀, with *Camponotus aethiops*, 21. v. 2005. Fig. 10: ♂, with 2 *Camponotus aethiops*, 24. v. 2006. Figs. 11–12: ♀, with *Camponotus aethiops*, Kalavrita (Peloponnese), 1200 m, 23. v. 2005. Fig. 13: ♂, with *Camponotus laconicus*, Pteri, 1200 m, 24. v. 2006. — Fig. 14: *Polyommatus thersites*, caterpillar on *Onobrychis ebenoides* with a *Camponotus sp.*, Pteri, 1200 m, 1. vi. 2005. — Figs. 15–19: *Glaucopsyche alexis*, caterpillars on *Onobrychis ebenoides*, all Pteri, 1200 m, 1. vi. 2006. Fig. 15: With 2 *Camponotus aethiops*. Fig. 16: With *Camponotus laconicus*. Fig. 17: With 2 *Camponotus aethiops*. Fig. 18: With ants *Crematogaster sordidula*. Fig. 19: With 2 *Camponotus aethiops*.
Parasitoids

Very few parasitoids have been obtained from reared caterpillars:

_Hyposoter notatus_ (Gravenhorst, 1829) (Hymenoptera, Ichneumonidae) from _Agrodiaetus_ larvae tentatively identified as _ripartii_ and _araoaniensis_. _Hyposoter notatus_ kills the caterpillar at the beginning or the middle of the last instar. The dead caterpillar dries and the parasitoid larva pupates within the mummified body of its host. The wasps hatched 12–13 days after the death of the caterpillar.

_Ichneumon exilicornis_ Wesmael, 1857 (Hymenoptera, Ichneumonidae) from _Agrodiaetus_ larvae. _Ichneumon exilicornis_ does not kill the caterpillar but pupates within the pupa of its host. Females attack grown caterpillars, prepupae and pupae. Our 3 specimens all hatched 14 days after the caterpillar pupated. Females are identical to those found in Central Europe but males differ in the shape of the tyloids and the darker colour of the 2nd and 3rd gastral tergites (K. Horstmann pers. comm. to M. Shaw).

_Cotesia_ cf. _astrarches_ (Marshall, 1889) (Hymenoptera, Braconidae) from _Polyommatus thersites_.

_Aplomys confinis_ (Fallen, 1820) (Diptera, Tachinidae) from one _Agrodiaetus_ larva.

Parasitism seems to vary greatly from one year to the following, especially the identity of the parasitoids. _Hyposoter notatus_ was the only parasitoid found in 2006 (3 specimens) whilst none were obtained in 2007 when we reared 3 _Ichneumon exilicornis_ and the single specimen of _Diptera: Tachinidae_.

In 2006, we collected _Agrodiaetus_ larvae in northern Greece. From caterpillars found on Mt. Smolikas (northern Epirus) at 1100–1300 m on 26. v. we reared the Braconidae _Cotesia astrarches_ and _Cotesia aff. tenebrosa_ Wesmael, 1837 and the _Tachinidae Aplomys confinis_. Caterpillars found at 1600–2100 m on Mt. Orvilos on 4. vii. and at 1600–1700 m on Mt. Phalakron (both in northeastern Macedonia) on 7. vii. produced _Ichneumon exilicornis_ and _Agrypon anomelas_ Gravenhorst, 1829.

We did not get any parasitoid from _G. alexis_ as we did not try to keep pupae in captivity throughout summer and winter.

Are ants efficient in protecting blue caterpillars against parasitoids?

The proportion of caterpillars attended by ants is definitely higher in the northern Peloponnese than on Mt. Smolikas at same level. The lowest number of attending ants was found in the grasslands above tree-line on Mts. Orvilos and Phalakron where the 2 larvae attended by ants each had a single small ant (_Plagiolepis vindobonensis_). The proportion of parasitized caterpillars follows an opposite trend. The samples from northern Greece are certainly smaller than for Mt. Klokos and percentages are given here only for comparison. This area was also explored at the end of the larval season and parasitized caterpillars are known to be late in comparison with healthy ones. This could have introduced a bias. For this reason, we started to collect caterpillars in the study area later in 2007 (26. v.–9. vi.) to get all the late caterpillars: there was no significant difference, with 4 parasitized caterpillars in 52 (8%). In northeastern Greece, on the contrary, where caterpillars were poorly attended by ants, the large _Ichneumon exilicornis_ seems to be a common parasitoid of _Agrodiaetus_, even as high up as 2100 m. The incidence of parasitized caterpillars given above certainly does not truly reflect the importance of
parasitism but only indicates a tendency as parasitoids which attack eggs or very young larvae as well as those attacking prepupae and pupae have not been sampled.

The 3 caterpillars parasitized by *Hyposoter notatus* in 2006 were all attended by small ants of the genus *Crema­marogaster*, respectively by 1, 1 and 2 ants when found, thus less than the average of small ants found with cater­pillars. As we have observed several times, large ants of the genus *Camponotus*, which attend 60% of the *Agro­diaetus* larvae on Mt. Klokos, are nervous and agressive towards other insects coming close to the caterpillar. On 30. v. 2006 we twice observed a *Camponotus* driving away a specimen of Hemiptera which had landed on the flower spike where an *Agrodiaetus* caterpillar was feeding. Such protective behaviour was also noted several time against bees. These large ants were also often trying to bite the ruler we used to measure the caterpillars. Small ants on the contrary often left the caterpillar or fell down to the ground as we took the plant in hand. Large ants can probably efficiently protect caterpillars from parasitoids which is perhaps not so much the case for smaller ants especially if they are in small numbers.

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**Table 5: Myrmecophily and parasitism of *Agrodiaetus* caterpillars in 3 areas in Greece.**

<table>
<thead>
<tr>
<th></th>
<th>Mt. Klokos</th>
<th>Mt. Smolikas</th>
<th>Mts. Orvilos &amp; Phalakron</th>
</tr>
</thead>
<tbody>
<tr>
<td>N caterpillars observed</td>
<td>393</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td>N caterpillars with ants</td>
<td>340</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>% caterpillars with ants</td>
<td>87</td>
<td>68</td>
<td>14</td>
</tr>
<tr>
<td>N caterpillars reared</td>
<td>120</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>N parasitized caterpillars</td>
<td>8</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>% parasitized caterpillars</td>
<td>7</td>
<td>14</td>
<td>43</td>
</tr>
</tbody>
</table>

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