

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

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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 Polyommata caterpillars feeding on the sainfoin *Onobrychis ebenoides* BOISS. & SPRUNER, their parasitoids and 10 species of attending ants.

Fünf Bläulinge an einer Pflanze: Interaktionen zwischen Polyommata (Lepidoptera, Lycaenidae), Ameisen und Raupenparasitoiden auf dem nördlichen Peloponnes (Griechenland)

Zusammenfassung: Feldstudien auf den Hängen des Berges Klokos (auf dem griechischen Peloponnes), unterstützt durch Zuchtversuche, zwischen 2005 und 2007 erbrachte Informationen über die Polyommata-Lebensgemeinschaft auf der Fabacee *Onobrychis ebenoides* BOISS. & SPRUNER, ihre Parasitoiden und 10 beteiligte Ameisenarten.

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 Lycaenidae 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 Lycaenidae 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 Polyommata 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 Polyommata 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.

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 Polyommata 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 Polyommata. 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 – *ripartii* (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. ripartii* and *A. aroaniensis*. The differences are more constant (75%) between *A. admetus* and *A. ripartii*, 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.

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, *Glauropsyche alexis* (PODA, 1761) (Figs. 15–19) and *Polyommatus thersites* (CANTENER, [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 Peloponnese. 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 thersites* 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* SM.), 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.

Glauropsyche 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 L₃ (less than 5 mm long), 23 L₄ (5–8 mm) and 30 L₅ (more than 8 mm). On 24. v. on 51 measured caterpillars there were 3 L₄ and 48 L₅. 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 Polyommatae, 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 Peloponnese, all the pupae kept under the same conditions (Table 1).

Table 1: Pupal development of *Agrodiaetus* species.

Species	n pupae	duration min.–max. [days]	average [days]
<i>A. ripartii</i>	33	16–23	20
<i>A. aroaniensis</i>	12	21–25	23
<i>A. admetus</i>	6	19–24	21

We have not looked for pupae in the field.

Another polyommatae 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

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

Ant species	Subfamily of Formicidae	Lycaenidae species				
		<i>G. alexis</i>	<i>P. thersites</i>	<i>A. admetus</i>	<i>A. ripartii</i>	<i>A. aroaniensis</i>
<i>Tapinoma simrothi</i>	Dolichoderinae	–	–	–	+	–
<i>Camponotus aethiops</i>	Formicinae	++	++	++	++	++
<i>Camponotus gestroi</i>	Formicinae	–	–	–	+	–
<i>Camponotus laconicus</i>	Formicinae	+	–	–	+	+
<i>Camponotus oertzeni</i>	Formicinae	–	–	–	+	+
<i>Camponotus piceus</i>	Formicinae	+	+	–	+	+
<i>Lepisiota melas</i>	Formicinae	–	–	–	+	–
<i>Plagiolepis pygmaea</i>	Formicinae	+	–	–	++	–
<i>Plagiolepis vindobonensis</i>	Formicinae	–	–	–	+	+
<i>Crematogaster sordidula</i>	Myrmicinae	++	++	++	++	++

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

No less than 10 species of ants have been found attending larvae of blues in the study area: 1 Dolichoderinae: *Tapinoma simrothi* KRAUSSE, 1909, 8 Formicinae: *Camponotus aethiops* (LATREILLE, 1798), *C. gestroi* EMERY, 1878, *C. laconicus* EMERY, 1920, *C. oertzeni* FOREL, 1889, *C. piceus* (LEACH, 1825), *Lepisiota melas* EMERY, 1915, *Plagiolepis pygmaea* (LATREILLE, 1798), *P. vindobonensis* LOMNICKI, 1925 and 1 Myrmicinae: *Crematogaster sordidula* (NYLANDER, 1849) (see Table 2).

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* (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 attractivity (DANIELS 2004).

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

	<i>G. alexis</i>		<i>P. thersites</i>		<i>Agrodiaetus</i> spp.	
	2006	2007	2006	2007	2006	2007
N caterpillars with ants	50	85	40	16	244	96
N caterpillars total	51	86	41	22	263	130
% with ants	98	99	98	73	93	74

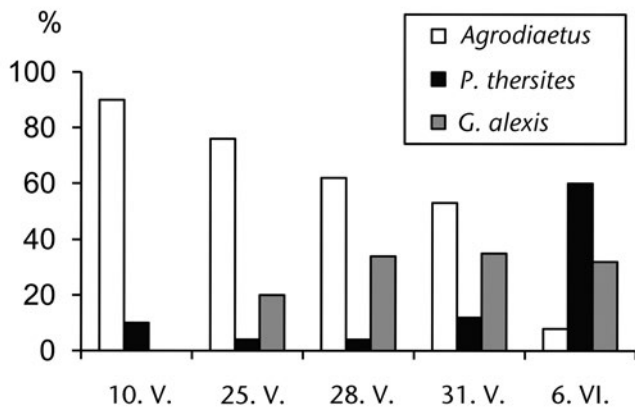
The size and the number of ants attending a caterpillar depends mostly on the size of the ants as shown in Table 4. They also depend on the size of the caterpillar (FIEDLER & HAGEMANN 1995, PETERSON 1995) but to a lesser extent as we observed large ants with small caterpillars several times. Though the 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 Polyommatae. Small ants are also on average more numerous with *G. alexis* (3.8) than with *Agrodiaetus* (2.7).



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 aroaniensis*, 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. vi. 2005. **Fig. 7:** ♂, with ants *Crematogaster sordidula*, 13. v. 2005. — **Figs. 8–13:** *Agrodiaetus ripartii*, L₄ (Figs. 8–9) and L₅ (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*.

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 Polyommatainae 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 Polyommatainae 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 laciniata* 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



Graph 1: Succession of the Polyommata caterpillars on *Onobrychis ebennoides* on Mt. Klokos in May and June.

are very busy around caterpillars until mid-June when they turn their attention to aphides on various plants and fewer ants are seen on sainfoins. The succession of the various species of blues (Graph 1) provides active caterpillars for at least 6–7 weeks: at the beginning of May, many young caterpillars of *Agrodiaetus* live with the last hibernated larvae of *P. thersites*. From the third week of May the number of *Agrodiaetus* larvae decreases whilst those of *G. alexis* become more numerous. In June, the latter species progressively disappears to pupate when the population of caterpillars of *P. thersites* produced by the first brood of butterflies is at its maximum.

Parasitoids

Very few parasitoids have been obtained from reared caterpillars:

Hyposoter notatus (GRAVENHORST, 1829) (Hymenoptera, Ichneumonidae) from *Agrodiaetus* larvae tentatively identified as *ripartii* and *aroaniensis*. *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 differs 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*.

Aplomya confinis (FALLEN, 1820) (Diptera, Tachinidae) from one *Agrodiaetus* larva.

Table 4: Number of attending ants per caterpillar. Ants of the genera *Tapinoma*, *Lepisiota*, *Plagiolepis* and *Crematogaster* are grouped together as “small ants” here.

N ants/caterpillar		1	2	3	4	5	6	7	8	9	11	12
<i>G. alexis</i>	small ants	3	4	4	6	2		2				1
	<i>Camponotus</i>	66	39	11	3	1						
<i>P. thersites</i>	small ants	3	4	2	2	1			1			
	<i>Camponotus</i>	34	5									
<i>Agrodiaetus</i> spp.	small ants	21	23	19	8	6	4	1	3	1	1	
	<i>Camponotus</i>	175	46	8								

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 *Aplomya 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

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

	Mt. Klokos	Mt. Smolikias	Mts. Orvilos & Phalakron
N caterpillars observed	393	40	14
N caterpillars with ants	340	27	2
% caterpillars with ants	87	68	14
N caterpillars reared	120	22	14
N parasitized caterpillars	8	3	6
% parasitized caterpillars	7	14	43

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 *Crematogaster*, 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 aggressive 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 times 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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