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Life cycle and growth pattern of the endangered myrmecophilous *Microdon myrmicae* (Diptera: Syrphidae)

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Abstract. In Europe there are only a few species of the syrphid fly *Microdon*, which live in the nests of various genera of ants. For most of these rare flies, details of their biology, larval behaviour and relationships with their hosts are still not yet well known. In this paper we present data on the life cycle, feeding behaviour and growth pattern of *Microdon myrmicae*, a social parasite of *Myrmica* ants and compare it with two species of *Maculinea* butterflies similarly parasitizing *Myrmica* ant colonies. *M. myrmicae* has three larval instars and overwinters as a third instar. Eggs and 1st instar larvae are ignored by ants, which indicate that they are "chemically insignificant". 2nd and 3rd instar larvae feed on small ant brood. *M. myrmicae* larvae grow rapidly from May to July and later in the year the host colony only serves as shelter for overwintering. Like *Maculinea alcon*, larvae of *M. myrmicae* are numerous in *Myrmica* nests and more numerous than those of *Maculinea teleius*. Since the larvae of *Microdon* feed on an abundance of young ant brood, they experience low level of scramble competition and although many may develop in an ant's nest they have probably little effect on host colony fitness.

INTRODUCTION

Numerous arthropods, such as beetles, butterflies and syrphid flies penetrate, inhabit and exploit ant societies (Hölldobler & Wilson, 1990; Thomas et al., 2005). Although it is estimated that there are about 10,000–20,000 species of social parasites of ant's (Thomas et al., 2005) details of the biology and life history traits are known for only a small number of species (Thomas et al., 2005). In Europe, one of the best-studied systems is that of *Maculinea* van Eecke 1915, butterflies and their *Myrmica* Latreille 1804, host ants (Settele & Kühn, 2009). Ants of this genus are also exploited by another social parasite, the syrphid fly *Microdon myrmicae* Schönrogge et al., 2002 (Schönrogge et al., 2002; Bonelli et al., 2011).

The life cycles of all known species of *Microdon* are invariably connected with ants. The fly larvae live inside ant colonies and exploit them as food resource and shelter (Elmes et al., 1999). Most species of *Microdon* live in tropical regions (Cheng & Thompson, 2008) but some are known to occur in the Palearctic (Andries, 1912; Doczkal & Schmid, 1999) and Nearctic (Duffield, 1981; Akre et al., 1988). In Europe there are relatively few species, i.e. *Microdon miki* Doczkal & Schmid 1999, *M. devius* (Linnaeus, 1761), *M. mutabilis* (Linnaeus, 1758), *M. myrmicae*, *M. analis* (Macquart, 1842) and *M. major* Andries, 1912 (Doczkal & Schmid, 1999; Schmid, 2004; Witek et al., 2011), although their number may be underestimated since social parasites are prone to cryptic speciation (Elmes et al., 1999; Schönrogge et al., 2002). Little is known about the life cycle, habitat preferences and interactions with host ants for most of European species of *Microdon*, with detailed information on host-ant specificity and habitat requirements known only for *M. mutabilis* (Elmes et al., 1999; Schönrogge et al., 2002, 2006, 2008). Schönrogge et al. (2002) separated *M. myrmicae* from *M. mutabilis* mostly on the basis of some morphological traits of the pupae and host ant specificity. *M. myrmicae* only exploits nests of *Myrmica* ants, whereas *M. mutabilis* is associated with colonies of *Formica lemanii* Bondroit, 1917 (Schönrogge et al., 2002). Recent investigations in various parts of Europe show that *M. myrmicae* uses mostly *Myrmica scabrinodis* Nylander, 1846 although some populations also successfully use colonies of other *Myrmica* species such as *M. gallienii* Bondroit, 1920 or *M. rubra* (Linnaeus, 1758) (Bonelli et al., 2011). Moreover, the same authors demonstrate that the occurrence of *M. myrmicae* is associated with wet, often temporarily waterlogged grasslands dominated by *Molinia* spp. There is no information, however, on larval development and the way they use resources in ant colonies. In this paper we present data on the life cycle, larval growth pattern and the potential influence of *M. myrmicae* on colonies of its host ant. This information will provide a better insight into the relationships of other European species of *Microdon* with their hosts.

MATERIAL AND METHODS

Study area

The life cycle and larval growth pattern of *M. myrmicae* were investigated in a population located in a wet meadow dominated by *Molinia coerulea*, at Caselette near Turin, in northern Italy (45°70′N, 07°29′E; 360 m a.s.l.). The site is also inhabited by the butterflies *Maculinea alcon* (Dennis & Schiffermüller, 1775) and *Maculinea teleius* (Bergsträsser, 1779), both of which are obligate parasites of *Myrmica* ants. The local host ant species of these three parasites is *Myrmica scabrinodis*, the only *Myrmica* species present at Caselette. The study site is in the NATURA-2000 site "Monte Musine-Laghi di Caselette" (IT1110081).

The life cycle of *Microdon myrmicae*

In 2007, 2009 and 2010 adults, larvae and pupae of *Microdon myrmicae* were collected from the field. At the end of May 2007, four adult females of *M. myrmicae* were collected and

TABLE 1. Summary of methods and results used for describing the life cycle and growth pattern of *Microdon myrmicae.*

METHODS	RESULTS
(1) May 2007: 4 adult females were collected in the field and reared in the laboratory where they laid their eggs on the surface of *Myrmica* nests.	(1) Eggs (109) and 1st instar larvae (19) behaviour and interaction with ants were observed in the laboratory.
(2) April and May 2008: searching for larvae/pupae/exuvia of *Microdon myrmicae*, *Maculinea alcon* and *Maculinea teleius* in *Myrmica* ant nests. 200 nests were examined.	(2) Estimation of median number of parasite larvae per host nest. 131 specimens of *M. myrmicae* were found in 30 *Myrmica* nests, 56 of *M. alcon* in 15 nests and 19 of *M. teleius* in 10 nests.
(3) September 2009: 10 larvae were collected in the field, taken to the laboratory, weighed and reared with *Myrmica* ants until spring 2010.	(3) Feeding behaviour and survival of 3rd instar larvae were observed in the laboratory and the results were used for describing life cycle.
(4) July–October 2010 and April–May 2011: 29 larvae and 4 pupae were collected in the field, taken to the laboratory, weighed and reared with *Myrmica* ants. Seven of the larvae collected in July were fed eggs, small and large larvae, and pupae of ants.	(4) Data on body mass of field-collected larvae and pupae were used to describe the growth of the larvae and life cycle. The feeding behaviour of the 7 larvae collected in July was recorded.

taken to the laboratory where they were kept in plastic boxes (24 × 5 × 16 cm) containing grass and colonies of *M. scabrinodis.* After oviposition, the development of the eggs and behaviour of the 1st instar larvae were observed, as well as the behaviour of the ant workers.

During September and October 2009, ten *M. myrmicae* larvae were collected and taken to the laboratory together with their host nests. The fly larvae were immediately weighed to the nearest 0.1 mg using a Precisa® digital balance. Each colony containing larvae was placed in a transparent plastic box (20 × 15 × 5 cm). Part of the box floor was covered with fine plaster, which was moistened with water to maintain a suitable humidity. Once a week, the ants were fed with glucose and frozen *Drosophila* larvae, and the behaviour of *M. myrmicae* larvae was observed for half an hour. *Myrmica* colonies were kept in the laboratory at a temperature of 20°C under a natural diurnal light cycle until May 2010.

In July 2010, ten small *M. myrmicae* larvae were found inside *M. scabrinodis* nests and taken to the laboratory. Seven of them were reared on a special food regime (see below) in order to observe their feeding behaviour and the other three were kept in plastic boxes together with their original host colonies. All these larvae were exposed to a natural light cycle and kept at a temperature of 20°C until they died.

Growth pattern and feeding behaviour of *M. myrmicae* larvae

In 2010 at the beginning of larval development *M. myrmicae* larvae were collected from the field on the following dates to evaluate their growth pattern and feeding behaviour: (i) 25/06 (no larvae), (ii) 05/07 (5 larvae), (iii) 16/07 (5 larvae), (iv) 27/07 (5 larvae), (v) 15/09 (5 larvae) and (vi) 14/10 (4 larvae). In 2011 samples were collected at the end of larval development as follows: (vii) 06/04 (5 larvae) and (viii) 12/05 (4 pupae). All larvae and pupae were taken to the laboratory and immediately weighed.

Seven *M. myrmicae* larvae found in July 2010 were also reared in small artificial *Myrmica* nests in order to observe their feeding behaviour. Each artificial nest consisted of a transparent plastic box (5.5 × 5.5 × 4 cm) with part of the floor of the box covered with fine plaster, which was moistened with water. Each *M. myrmicae* larva was reared in a separate box containing: (i) five *Myrmica* workers, (ii) two ant eggs, and (iii) two small, two medium-sized and two large ant larvae. Both the ant workers and ant brood were from the same nest the *Microdon* larva was collected from. Fresh food (ant eggs and larvae) was offered and replaced every third day. Observations were carried out until the death of each *M. myrmicae* larva.

Number of social parasite larvae in *Myrmica* nests

Between the end of April and mid May 2008, *Myrmica* ant nests were opened and searched for evidence of *Microdon myrmicae* and *Maculinea* butterflies. At that time of the year the expectation is that there will be fully grown larvae, pupae, or fresh exuviae of *M. myrmicae* and medium sized larvae of *Maculinea* butterflies in the ant nests.

Summary of the methods used are presented in Table 1.

RESULTS

The life cycle of *M. myrmicae*

In the laboratory a total, 109 eggs (Fig. 1A) were laid on the outer surface of *Myrmica* nests by four females of *M. myrmicae*. The *M. myrmicae* eggs were ignored by *Myrmica* ants. Two weeks after oviposition, 1st instar larvae (Fig. 1B) started to emerge and quickly entered the ant nest. In no case was any interaction between *M. myrmicae* larvae and *Myrmica* ants detected. All laboratory-reared 1st instar larvae died after a few days: the maximum observed lifespan was 13 days. Field-collected (July 2010) 2nd instar larvae (Fig. 1C) actively moved into artificial ant nests and on a few occasions feeding behaviour was observed (described below). Laboratory observations indicate that these 2nd instar larvae moulted to the last, 3rd instar larva in July (Fig. 1D). Ten *M. myrmicae* 3rd instar larvae found in September 2009 were weighed (134. 9 ± 38.7 mg) (mean ± SD) and kept in the laboratory in nests of *M. scabrinodis* as described above. In no case were they observed feeding and they remained immobile in the outer parts of the artificial nest. Nine of the ten *M. myrmicae* larvae survived the winter. One pupated at the end of February and the others in March and April (Fig. 1E), and on average adult flies emerged after three weeks (Fig. 1F).

Growth pattern and feeding behaviour of *M. myrmicae* larvae

No larvae were found at the end of June because at that time they are too small to be detected in the field. The mean body mass (± SD) of *M. myrmicae* larvae found at the beginning of July was 10.1 ± 5.48 mg. The smallest larva weighed 4.7 mg and the heaviest 16.4 mg. The first big increase in body mass was recorded in the second half of July and at the end of this month the larvae weighed 89.9 ± 16.43 mg (Fig. 2). During the next two months (August and September) larvae continued to

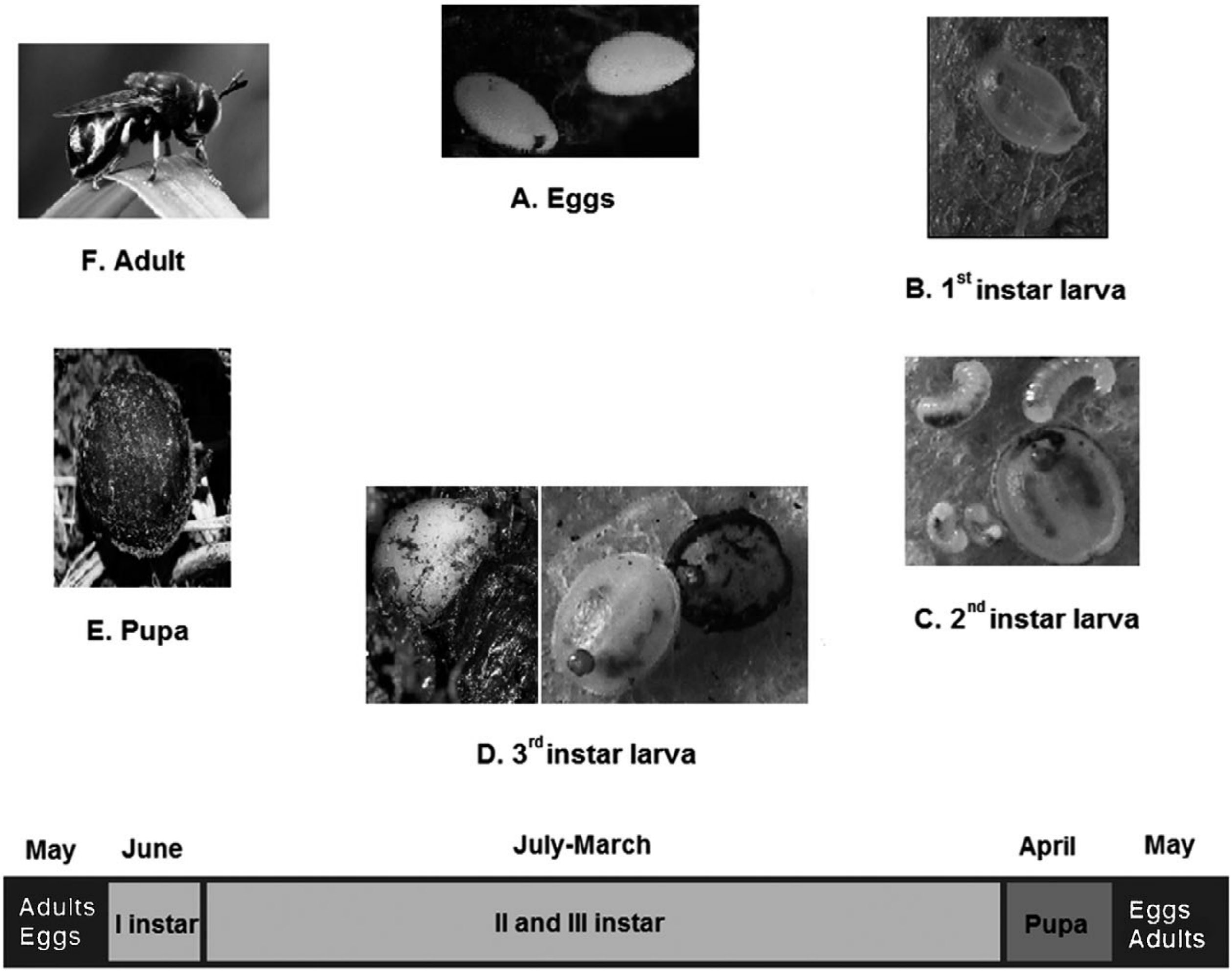

Fig. 1. The life cycle of *Microdon myrmicae*. Adult females (F) lay their eggs (A) on the surface of *Myrmica* nests in May. 1st instar larvae (B) hatch from eggs in June and then moult to 2nd instar larvae (C). 3rd instar larvae (D) spend about 8 months in ants' nest and in April of the following year they pupate (E).

grow rapidly and in the middle of October they weighed on average 125.4 ± 28.24 mg and the heaviest 157.4 mg. Larvae collected after winter were slightly lighter than in the previous autumn with a mean body mass of 113.9 ± 20.77 mg. The average pupal body mass was 100.0 ± 16.1 mg (Fig. 2, last measurement).

The seven laboratory-reared *M. myrmicae* larvae were recorded feeding on 12 occasions, mostly eating small *Myrmica* larvae (9 cases) or medium-sized ant larvae (3 cases) but none fed on ant eggs or large ant larvae.

Number of larvae of social parasites recorded in *Myrmica* nests

In total, 200 nests of *M. scabrinodis* were investigated of which 30 contained larvae of *M. myrmicae*, 15 *Maculinea alcon* larvae and 10 *Maculinea teleius* larvae (Table 1). The highest median number of larvae of *M. alcon* per nest was three (with quartiles of one and four; 1, 4). For *M. myrmicae* the corresponding values were 2.5 (1, 4) and for *M. teleius* 1 (1, 2). There were significantly more *M. myrmicae* larvae per *Myrmica* nest than of *M. teleius* (Mann-Whitney test, N = 41, U = 102.5, p = 0.05), but no difference in the numbers of *M. myrmicae* and *M. alcon* larvae per host colony (Mann-Whitney test, N = 45, U = 208, p = 0.67). The maximum number of larvae found in the same *Myrmica* nest was 27 *M. myrmicae*, 13 *M. alcon* and 5 *M. teleius*.

DISCUSSION

We describe the full life cycle of one European species of *Microdon* and shed some light on the potential effect it has on the fitness of the host-ant colony. *M. myrmicae* passes through three instars and overwinters as 3rd instar larvae. Our observations show that *M. myrmicae* probably has one generation per year. Immediately before pupation, all larvae were the same size, which strongly suggests that they take a year to develop. These two traits (univoltinism and length of larval development) seem to vary among species of *Microdon* since, for example, *M. fuscipennis* (Macquart, 1834) has more than one generation per year, whereas *M. xanthopilis*, Townsend, 1895 is similar to *M. myrmicae* in only having one (Akre et al., 1973; Duffield, 1981). Interestingly, Schönrogge et al. (2000) demonstrate that English populations of *M. mutabilis* and *M. myrmicae* include both slow- and fast-developing larvae, which means that some of them complete their development in one and others in two years. This is similar to what is observed for some *Maculinea* populations, where larval polymorphic development seems to be restricted to some northern and mountainous sites as it has not been recorded in the central and southern parts of their ranges (Nowicki et al., 2009). *M. myrmicae* populations may also vary

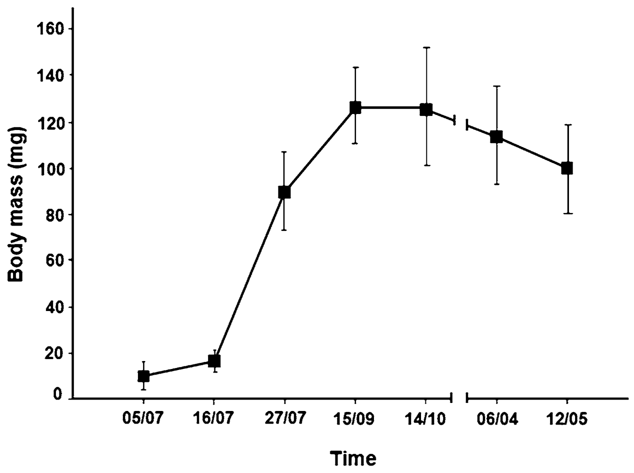

Fig. 2. Trend in the body mass (mean ± SD) of *M. myrmicae* larvae collected in the field from the beginning of June 2010 till April 2011. The last measurement is the mean body mass of the *M. myrmicae* pupae collected in May 2011.

with respect to larval polymorphic development, since in some northern areas such as England and Poland (Witek, unpubl. data), two larval cohorts are found, whereas in the southern populations the larvae take only one year to complete their development. Developmental polymorphism may represent an adaptation to life in more unpredictable habitats (Witek et al., 2006).

Myrmica workers did not respond to the eggs and 1st instar larvae of *Microdon* even when the latter moved towards the nest entrance and entered the nest. This suggests that both the eggs and larvae use "chemical insignificance" to avoid detection by the host ants (Lenoir et al., 2001). In contrast, Duffield (1981) records that 1st instar larvae of *M. fuscipennis* were often detected by workers of their host ant, *Iridomyrmex pruinosus* (Wheeler, 1913) and carried out of the colony. Garnett et al. (1985) records that in three North American species of *Microdon* (*M. albicomatus* Novak, 1977, *M. cothurnatus* Bigot, 1884, and *M. piperi* Knab, 1917) the host ants actively transport 1st and the 2nd instar larvae into the deeper parts of the nest, as well as from one brood chamber to another, suggesting that these *Microdon* larvae are well integrated with their host colony and use either chemical camouflage or mimicry.

The percentage mortality of *Microdon* 1st instar larvae recorded in this study and by Duffield (1981) in the laboratory was very high. The behaviour of the 1st instar larvae in both this and Duffield's study was very similar in that they were very mobile, moved into the deep parts of the colony and were not observed to feed or be fed by the ants. The feeding behaviour of the 2nd and 3rd instar larvae of *M. myrmicae* is also similar to that recorded for *M. fuscipennis* (Duffield, 1981). The fly larvae consumed small- or medium-size ant brood and avoided feeding on large ant larvae or pupae. Interestingly, our field data indicate that *M. myrmicae* larvae grow quickly during the first four months of their life inside host colonies and reach maximum body mass in September and October, just before overwintering. Moreover, none of the 3rd instar larvae collected in September was seen to feed. Both of these observations suggest that *M. myrmicae* larvae eat only at the beginning of their development in a host colony, whereas during winter and the following early spring they use ant nests only for shelter. The fact that *M. myrmicae* feeds only on the small or half-grown ant brood may have important consequences for the fitness of the host colony, as well as decreasing inter-specific competition and increasing survival of larvae coexisting within the same host colony. Comparing the median number of *M. myrmicae* larvae found at the end of their larval development with those of two *Maculinea* butterfly species, with different feeding strategies, in the same host colony (Thomas & Elmes, 1998) indicates that *Microdon* is more similar to *M. alcon* (a "cuckoo species" fed by trophallaxis) than *M. teleius* (a predatory species). Although *M. myrmicae* is a predator, as is *Maculinea teleius*, and feeds directly on ant brood, it is not subject to the same high level of scramble competition as the butterfly larvae. In this study the highest number of larvae of *M. myrmicae* recorded was found in a single host colony and all were large enough to complete their life cycle. The high percentage of larvae of *Microdon* that survive is probably due to fact that they feed only on small ant larvae, whereas predatory species of *Maculinea* prefer to eat large ant brood (Thomas & Wardlaw, 1992). Additionally, because *M. myrmicae* feeds mostly on the early stages of ant brood it possibly has a smaller negative effect on the host colony than predatory *Maculinea*. High densities of predatory *Maculinea* may induce the ant workers to desert their nest, which they do in the absence of brood (Thomas & Wardlaw, 1992).

Differences in the life cycles of the various species of *Microdon* may be due to their different host specificities. A better understanding of the biology of other European species of *Microdon*, coupled with more detailed studies of their chemical and acoustical adaptations, will probably shed some additional light on social parasite-host interactions.

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