

Life-history traits in a parasitoid dipteran species with free-living and obligate parasitic immature stages

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Abstract. The robber fly *Mallophora ruficauda* Weidemann (Diptera: Asilidae) is an important pest of apiculture in the Pampas of Argentina. As adults, they prey on honey bees and other insects, whereas the larvae are ectoparasitoids of Scarabaeidae grubs. Females of *M. ruficauda* lay eggs in grassland where the larvae drop to the ground after being wind-dispersed and burrow underground searching for their hosts. A temporal asynchrony exists between the appearance of the parasitoid larvae and the host, with the parasitoid appearing earlier than the host. The present study investigates whether a strategy of synchronization with the host exists in *M. ruficauda* and determines which of the larval instars are responsible for it. Survival patterns and duration of the immature stages of the parasitoid are investigated to determine whether there is a modulation in the development at any time that could reduce the asynchrony. Experiments are carried out to determine the survival and duration of free-living larval stadia in the absence of cues associated with the host. It is established that the first instar is capable of moulting to the second instar without feeding and in the absence of any cues related to the host, a unique event for parasitoids. Also, the first instar of *M. ruficauda* moults to the second stage within a narrow temporal window, and the second instar never moults in the absence of the host. After parasitizing a host, the second instar has the longest lifespan and is the most variable with respect to survival compared with the rest of the instars. All larval instars, except for those in the last (fifth) stadium, have a similar rate of mortality to that of second-instar larvae. Additionally, it is established that the host is killed during the fourth (parasitoid) stadium and that the first- and fifth-larval instars develop independently of the host. Finally, possible mechanisms that could aid in compensating for the asynchrony between the parasitoid and the host, promoting the host–parasitoid encounter, are discussed.

Key words. Asilidae, asynchrony, *Mallophora ruficauda*, Scarabaeidae, survival.

Introduction

It is accepted that natural selection often determines which individuals survive and reproduce; however, the actual number of individuals that survive is generally determined by external limiting factors, such as food, space or predation, influencing the population dynamics of a particular system (Saccheri &

Hanski, 2006). Population dynamics models in parasitoid–host interactions are proposed mainly from studies on the effects of host-feeding on nutrient allocation decisions (Collier *et al.*, 1994; Briggs *et al.*, 1995; Casas *et al.*, 2005; Pelosse *et al.*, 2007) and host location (Godfray *et al.*, 1994; Hassell, 2000). Although much attention focuses on spatial heterogeneity, far fewer studies deal with temporal inhomogeneity (Godfray *et al.*, 1994). A consequence of temporal asynchrony is that part of the host population is preserved from the risk of being parasitized by the creation of a partial refuge effect. In this context, factors involved in the synchronization of host–parasitoid systems are important as determinants of resource availability.

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There are several strategies for synchronization between hosts and parasitoids. It is very common for parasitoids to enter diapause or a resting stage when hosts are unavailable (Godfray, 1994). Some parasitoids respond passively to the host's physiology mainly by detecting changes in the level of host hormones associated with diapause and, as a result, the parasitoid remains in the first larval stage if the host enters diapause (Fisher, 1971; Tauber *et al.*, 1983, 1986). However, some parasitoid immature stages adjust their development to that of the host or, alternatively, actively regulate the development of the host (Fisher, 1971; Vinson, 1976; Godfray, 1994; van Nouhuys & Lei, 2004). Some endoparasitoids, mainly koinobionts, slow down their development until the host is fully grown (Godfray, 1994). Although there are many studies dealing with the mechanisms that underlie host location and synchronization of parasitoid development with that of its host, these are restricted mainly to parasitoid species (endoparasitoids) that oviposit into the host. Far less is known about host location by parasitoid species in which eggs are laid away from the host (Godfray, 1994). In this group, the parasitoid females do not secure the encounter between the progeny and the host, and the parasitoid larvae must find the host by means of active searching or foresis (Godfray, 1994). Strategies of synchronization with hosts are well studied among hymenopterans (Askew, 1971; Tauber *et al.*, 1983, 1986; Danks, 1987; Bonsall & Hassell, 1999; van Nouhuys & Lei, 2004), although there is less information available from other orders (Vinogradova, 1991). In the Asilidae (Diptera) in particular, there are no studies on the mechanisms underlying the synchronization of host-seeking larvae with their hosts.

Mallophora ruficauda Wiedemann (Diptera: Asilidae) is a parasitoid robber fly endemic to the Pampas region of Argentina that inhabits open grasslands near bee farms (Rabinovich & Corley, 1997). Adult *M. ruficauda* are predators of foraging honey bees and other Hymenoptera, whereas the larvae are ectoparasitoids (i.e. insects that feed on killed or paralyzed hosts) of scarab beetle larvae (Coleoptera), commonly known as white grubs. The potential of robber fly larvae as biological control agents for white grubs is the subject of some studies. Wei *et al.* (1995) demonstrate that *Promachus yesonicus* Bigot can reduce white grub densities up to 99% in experimental fields in China. By contrast, Castelo & Corley (2004) find an inverse density-dependent relationship between white grub populations and parasitism by *M. ruficauda*, suggesting that the fly cannot control white grubs at higher densities effectively.

Mallophora ruficauda has an annual life cycle with five immature instars prior to the pupal stage. Female *M. ruficauda* lay egg clutches on tall grasses or artificial supports such as wire fences (Copello, 1922; Castelo & Corley, 2004; Castelo *et al.*, 2006). Oviposition occurs from mid December until mid March, with a maximum peak during February (Castelo, 2003). Selection of oviposition height by the parasitoid female contributes to larval dispersal and, as a result, parasitism success is maximal when egg clutches are placed on substrates with a height in the range 1.25–1.50 m (Castelo *et al.*, 2006). After hatching, larvae are wind-dispersed and, once on the ground, start digging in the soil searching for their host, mainly

third-instar larvae of *Cyclocephala signaticollis* Burmeister (Coleoptera: Scarabaeidae) (Castelo & Capurro, 2000; Castelo & Corley, 2010). First-instar larvae do not feed but search for a suitable microhabitat where they moult into the second instar. During this stadium, host location and parasitism takes place (Crespo & Castelo, 2008). Once parasitism occurs, the parasitoid larva remains attached to its host during the winter as a second-instar larva. Then, in spring, the parasitoid larva develops rapidly and, after 1 month, reaches the fifth and final larval stage before the host pupates. In many ways, the second-instar larvae of *M. ruficauda* behave similarly to the first-instar larvae of every other known parasitoid in which oviposition is away from the host, in that this is the stage where host location, acceptance and intraspecific competition occurs. Second-instar individuals of *M. ruficauda* may therefore be expected to have the highest mortality risk. Egg hatch in *M. ruficauda* commences 7 days after oviposition (Castelo & Corley, 2004). The parasitoid eggs start to hatch in mid December, although the preferred host stage (i.e. third-instar larvae of the white grub) is not available until mid February. The question that arises is how the larvae of *M. ruficauda* survive until the preferred host stage becomes available. The present study investigates the life-history traits that larval stages of *M. ruficauda* use to survive until the host is available. In addition, the length and survival of each larval stage and pupa are investigated. It is hypothesized that the parasitoid synchronizes its development with that of the host by extending the larval stage that has to locate hosts.

Materials and methods

Insects

Mallophora ruficauda eggs were collected from herbaceous vegetation in grasslands located in Moreno (34°46'S, 58°93'W), Pilar (34°28'S, 58°55'W) and Mercedes (34°65'S, 59°43'W). The three localities are associated with apiaries in Buenos Aires province, Argentina, and were sampled during the summers (January to March) of 2006 and 2008. Egg clutches were collected by cutting the branches to which they were attached, and were stored individually in 30-mL polythene centrifuge tubes until the eggs started hatching. Immediately after egg hatching, neonate larvae were separated individually in 1.5-mL microcentrifuge tubes with a moistened piece of filter paper as substrate. Drops of clean water were added, when necessary, to maintain 100% humidity inside the tubes and to avoid dehydration of the larvae. Tubes were stored in complete darkness at room temperature in the range 18.6–29.8 °C until larvae reached the specific age classes and stadia for use in the experiments.

The host *C. signaticollis* was collected as third-instar larvae from soil samples in the same localities as the parasitoid egg clutches. The larvae were collected in autumn (March to June) of 2006 and 2008 in grasslands near bee hives where robber fly activity had been observed during previous summers. A random sampling method was performed and larvae were collected by digging the soil to a depth of 0.30 m at each

site. Individual larvae were identified to species level in the laboratory by means of a taxonomic key (Alvarado, 1980). The number of larvae of *M. ruficauda* attached externally to the host cuticle per beetle larva was also recorded using a magnifier lens ($\times 16$). Hosts were then classified according to the degree of parasitism. Unparasitized hosts were used for artificial parasitism, whereas already parasitized hosts were studied from that point onward. In this way, parasitoid larvae of a known time subsequent to parasitism were obtained. Hosts were kept individually at room temperature in black tubes filled with soil, and fed weekly with fresh pieces of carrot.

Instar-specific survival rates and stadium duration in the presence and absence of hosts

To determine instar-specific survival rates and the duration of each stadium, neonate larvae were placed individually in flasks (radius 3 cm, height 5 cm) with a moistened piece of filter paper as substrate at the bottom. Fifty neonate larvae per day were used for ten consecutive days to obtain larvae that emerged when the oviposition rate was higher ($n = 500$). During the experiment, parasitoid larvae remained in the absence of any cues associated with the host or the host environment. Flasks were stored in complete darkness at temperatures (range 18.6–29.8 °C, mean temperature = 27.4 °C) similar to those in the wild where the larvae were collected. Each individual was observed daily and records were kept of whenever ecdysis or death took place. The experiment was concluded on the death of the last larva.

To determine the survival and duration of the larval stadia associated with the host, parasitized hosts collected from the field were used and observed weekly, again recording whenever ecdysis or death took place. The sampling frequency was deliberately lower compared with the previous experiment because it has been noted that disturbance of the host increases their risk of mortality after parasitism occurs (M. K. Castelo, unpublished data). A total of 111 parasitized hosts in different stages of parasitism were collected (as second-, third- or fourth-instar larvae). In addition to the field-collected parasitized hosts, 45 hosts were parasitized in the laboratory. One second-instar larvae was presented to each unparasitized white grub and, after 1 week, the occurrence of parasitism was checked. In those cases where parasitism was successful, each individual was observed weekly as described above. The experiment was concluded when all parasitoid larvae had either died or an adult

fly had emerged. To determine whether the parasitoid larva can survive in absence of the host, the fate of larvae of all instars was analyzed for those cases when the host eventually died. The same individuals were used as in the previous studies, although only those where the host died during the follow-up were analyzed. When a dead host was detected, a record was made of whether the parasitoid larva had moulted to the next stadium or had died along with the host.

Statistical analysis

The time until death or moulting of every individual was measured to obtain survival and duration curves for each larval stadium and for the pupa. A Kaplan–Meier survival analysis was performed (Rosner, 1995). The proportion of individuals that survived each stadium was calculated and compared by means of a Tukey's honestly significant difference test (Zar, 1996). Taking into account that only the second instar is capable of host location, it was considered that those first stadium individuals that did not moult would not be able to parasitize a host (Crespo, 2007; Crespo & Castelo, 2008). Hence, because larvae that failed to moult before the tenth day did not moult at all, the survival of the first instar and the duration of the first stadium were analyzed only for the period comprising from egg hatch until the tenth day. Finally, the proportion of parasitoid larvae that survived whenever the host died was calculated for each stadium and the mortality in each stadium was compared with that of the second stadium.

Results

Instar-specific survival rates and stadium duration in the presence and absence of hosts

It was found that almost 85% of first stadium larvae are capable of surviving and moulting to the second stadium in the absence of the host or cues related to it. Under laboratory conditions, individuals reached the second stadium mainly on the day 7 after hatching (Table 1).

None of the second-instar larvae that were reared in absence of cues related to the host, moulted to the third instar ($n = 360$; Table 1, Fig. 1). Because all larvae failed to moult to the next stage, the duration and the survival curves are the same in this

Table 1. Stadium duration and survival of different larval instars and pupal stage of *Mallophora ruficauda* reared in the absence and presence of hosts.

Stadium	Moulting peak (days)	Median stadium duration (lower, upper quartile) (days)	Percentage of individuals surviving	<i>n</i>	Description
I	7	7 (6, 9)	84.9	424	Larvae reared in absence of host
II	—	32 (19, 51)	0	360	Larvae reared in absence of host
II	109	109 (43.2, 158)	76.7	58	Larvae reared on both artificially and naturally parasitized hosts
III	4	4 (4, 8)	71.9	57	Larvae reared as above
IV	4	4 (4, 12.66)	70.4	54	Larvae reared as above
V	5	5 (4, 11)	100	43	Larvae reared as above
Pupa	24	24 (20.5, 29)	86.1	43	Pupae reared from parasitized larval instars

Table 2. Percentage of larvae of *Mallophora ruficauda* surviving when the host died.

Stadium	Percentage of individuals surviving after the host's death	<i>n</i>	Description
II	0	71	Larvae reared on both artificially and naturally parasitized hosts
III	0	1	Larva collected from the field already parasitizing a host
IV	92.7	41	Larvae reared on both artificially and naturally parasitized hosts from previous instars

No subsequent hosts or food were available for the parasitoids once the host died.

case. This shows that the second-instar larvae must locate a host within this stadium to continue development, and that the larvae can wait for a host in the soil for up to 50 days (Table 1, Fig. 1).

Survival and duration in the different stadia (II–V) were very different. Using an artificial parasitism procedure of unparasitized hosts exposed to second-instar free-living larvae of the parasitoid, larvae that reached the third instar were obtained ($n = 45$). Naturally parasitized hosts of various instars collected from the field ($n = 30$) were also used in these experiments.

The only way to determine the duration of the second stadium once parasitism has occurred is to parasitize hosts experimentally, and manipulation such as this could influence the natural process. To rule out differences in the survival dynamics between hosts parasitized artificially ($n = 45$) and naturally ($n = 12$), a survival analysis of the second stadium was performed. This showed that both groups were similar with respect to survival times and thus were analyzed as a single set of data (Gehan's Wilcoxon test, test statistic = 0.306, $P = 0.759$; $n = 58$; Table 1). The average duration of the second stadium, taking into account both the free-living and parasitic phases, was 141 days.

For the third, fourth and fifth stadia, the median duration was less than 1 week under laboratory conditions, and the variability was less than in the first and second stadia ($n = 54$; Table 1). Interestingly, the parasitoid kills the host during the fourth parasitoid stadium before ecdysis to the final stadium occurs. The fifth larval stadium yielded results similar to the previous two stadia. However, all individuals pupated; thus, the fifth stadium had the lowest mortality ($n = 43$; Table 1). Finally, for the pupal stage, on average, adults emerged 24 days after pupation ($n = 43$; Table 1).

Finally, the proportion of larvae that died in each stadium was compared with respect to the second stadium to determine whether the latter had the higher risk of mortality. Differences were found between different stadia (i.e. first, third, fourth, fifth and pupa; Tukey's honestly significant difference test, $P < 0.001$). The fifth stadium had a lower mortality than the second stadium (Dunnett test: $Q_5 = 2.98$, $P < 0.05$). For

the remaining stadia and the pupal stage, no differences with respect to the second stadium were found (Dunnett test: $Q_1 = -0.63$, $P > 0.05$; $Q_3 = 0.72$, $P > 0.05$; $Q_4 = -1.92$, $P > 0.05$; $Q_{\text{pupa}} = -0.30$, $P > 0.05$). In those cases where the host died before the parasitoid larva moulted to the next stadium, the results obtained showed that development in different stadia of the parasitoid was directly related to the host, although some could still complete their development even when the host died. For the second stadium, no larvae completed their development if the host died, indicating that, at this point, the host must be alive for the parasitoid to moult ($n = 71$; Table 2). During the third stadium, 100% of the parasitoid larvae died when the host died, indicating that the host must be alive for the parasitoid to continue its development ($n = 1$; Table 2). Some 36.3% of the hosts died during the fourth stadium of the parasitoid ($n = 41$), although 92.7% of parasitoids survived and moulted to the next stadium under these conditions (Table 2). This shows that the fourth stadium of the parasitoid is no longer strictly dependent on the host. Finally, all parasitoid larvae that reached the fifth stadium survived further and pupated ($n = 43$; Table 1). Furthermore, no hosts were alive when the parasitoid larvae reached the last larval stadium, demonstrating that, similar to first-instar larvae, fifth-instar larvae do not require a live host.

Discussion

In the present study, the survival patterns and duration of all immature stages of *M. ruficauda* are described, and the strategy of synchronization with the host is investigated. In the absence of the host, larvae of *M. ruficauda* are able to reach the second stadium, apparently representing a unique event amongst parasitoids. However, if no parasitism occurs during the second stadium, the parasitoid dies. The third instar is also an obligate parasitoid, requiring a host to survive. Once the parasitoid reaches the fourth stadium, the association with the host is not so tight and, after a period of association, the parasitoid larvae can still survive and reach the final stadium if the host dies. It is during the fourth stadium that the parasitoid kills the host. The fifth-instar larvae of the parasitoid can be independent of the host and pupate after a short period of time. It can only be determined that larvae in these stadia moult within 1 week and no exact value for this duration is obtained in the present study. Although the duration of the different stadia is approximate, the huge difference in duration between the second (mainly searching) and the remaining stadia (linked to host) is established. Overall, the survival is quite low. From a sample of 100 individuals, only 15% reach the adult stage (Fig. 2). Furthermore, the highest mortality of individuals occurs during the second stadium.

The duration of second stadium is the longest and most variable of all. Although it is during this stadium that host location, parasitism and intraspecific competition occur, no difference in survival compared with the other stadia is found, except for the fifth stadium. The fifth stadium shows the lowest mortality of all immature stages, probably because of its independence with respect to the host. This indicates that

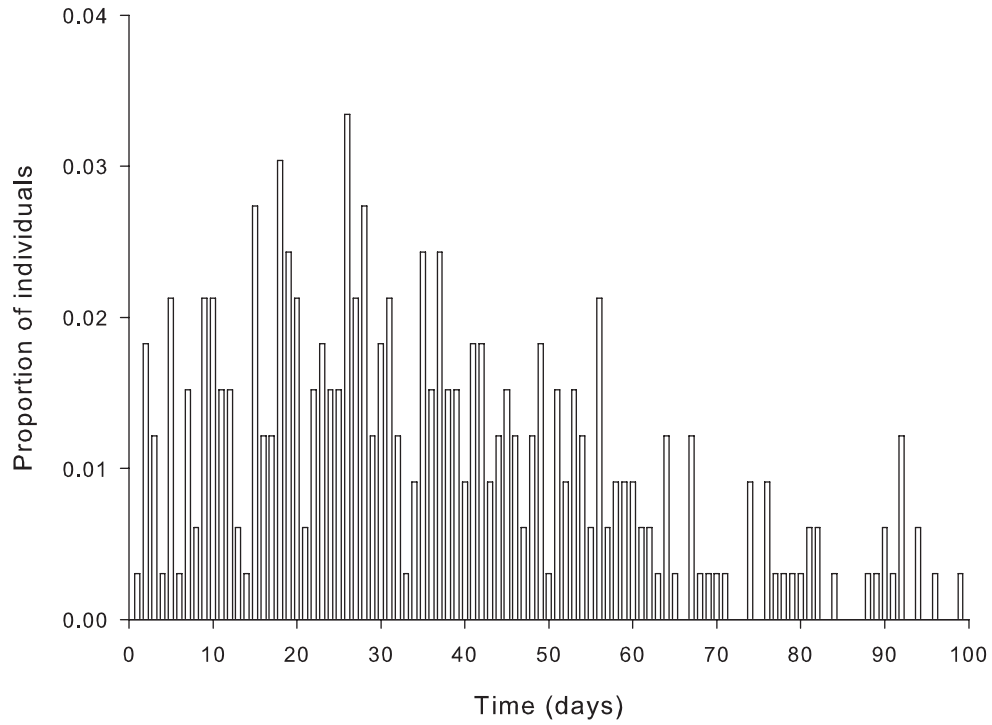


Fig. 1. Frequency distribution showing survival of second instar larvae of *Mallophora ruficauda* that failed to parasitize a host. None of the larvae under this condition reached the third stadium; they died as second-instar larvae.

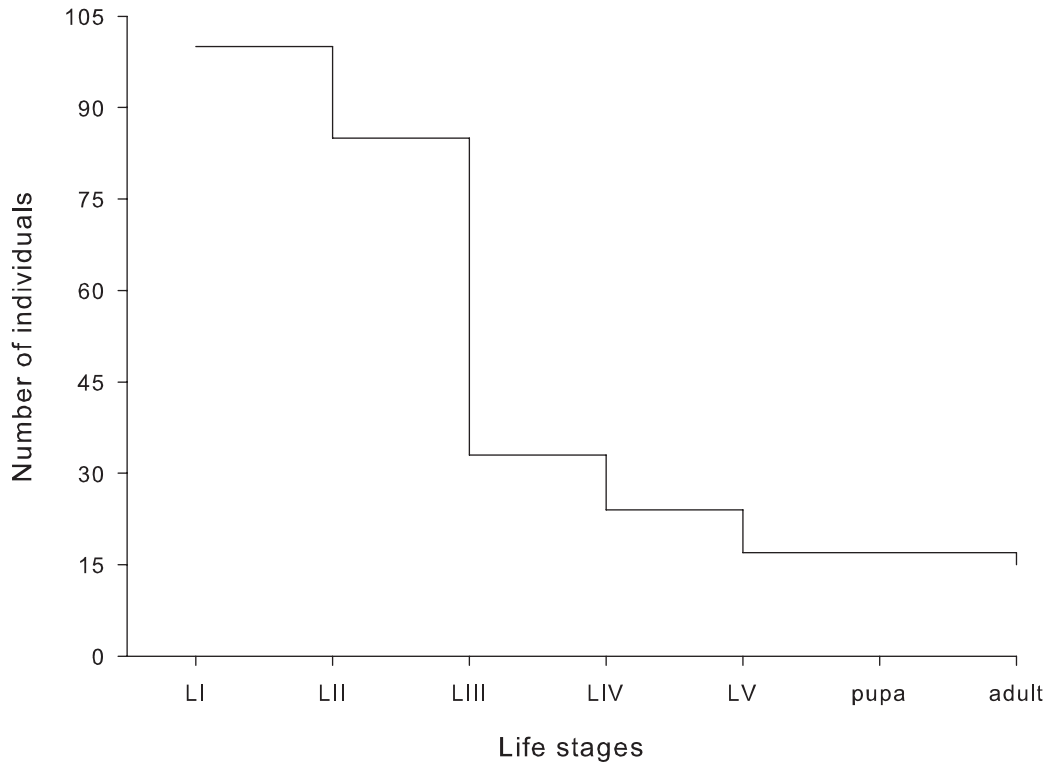


Fig. 2. Number of individuals of *Mallophora ruficauda* surviving from egg hatch to adult stage. Data are derived from the survival for each period of development. Larval instars: LI, first; LII, second; LIII, third; LIV, fourth; LV, fifth; pupa, pupal stage; adult, adult stage.

mortality is similar among the stadia; however, the artificial nature of the experiment may influence these results. Regarding the synchronization strategy, the second instar appears to be capable of modulating its development; thus, *M. ruficauda* regulates its own timing to that of the host by an extended period of survival in the absence of the host.

In all other parasitoids studied to date, host location and parasitism for those with a free-living larval stage are always undertaken by the first-instar larva (Godfray, 1994; Feener & Brown, 1997; Brodeur & Boivin, 2004). In *M. ruficauda*, however, the first-instar larva can complete its development using its own reserves and the second-instar larvae appear to behave in a similar way to the first instars of other parasitoids. Most other larvae of the genus *Mallophora* are predators, perhaps indicative that the first stadium is only a transition state between the egg and the host-seeking stadium, during which the microhabitat of the host is located. In this context, it appears advantageous that, for parasitoids with host-seeking larvae, the stadium in which host location and parasitism takes place should have a long duration and high rate of survival. In this context, it is noticeable that, in parasitoids where the adult oviposits directly on the host, the eggs or pupa comprise those stages with a high survival (Godfray, 1994).

In *M. ruficauda*, the second instar can survive for many days using its own reserves but depends on parasitizing a host to complete its development. Furthermore, the variability in the duration of this stage is higher than all the larval stadia and the pupal stage. Although this result may not be surprising, it is a central issue for the population dynamics of this species because it is during the second instar when some of the steps (i.e. host location and acceptance) that are necessary for a successful parasitization must occur (van Alphen & Vet, 1986; Brodeur & Boivin, 2004). Efficient host location is highlighted as being central to the persistence of interacting populations because it may modify the abundance of hosts and parasitoids (Chesson & Murdoch, 1986; Pacala *et al.*, 1990; Godfray *et al.*, 1994; Hassell, 2000). Besides being influenced by intrinsic factors such as the strategies used for host location, the abundance of hosts and parasitoids is also modified by heterogeneity (i.e. spatial and temporal) and can promote the persistence of interacting populations (Wiegand & Wissel, 1994; Briggs & Godfray, 1995; Briggs & Hoopes, 2004; Schley & Bees, 2006).

Temporal asynchrony (i.e. a mismatch between the adult parasitoid and the main peak of the appearance of hosts) might be a stabilizing factor for interacting populations because it could influence the degree of risk of parasitism among individual hosts by introducing a temporal refuge from parasitism (Godfray *et al.*, 1994). Although some hosts might benefit from this asynchrony (i.e. they might escape from parasitism), some overlap with the parasitoid populations still occurs. For this to occur, both the parasitoid and host must coexist spatially and temporally. The most common way in which parasitoids overcome this time gap is by diapause (Godfray, 1994). *Mallophora ruficauda* appears to employ a different strategy and overcomes this time gap as a result of a high survival during the second host-seeking stadium.

Larvae of this parasitoid are described by Musso (1981) as a planidium with a very high mobility and, hence, with a high metabolism. The only energy available for the maintenance and survival of the larva comes from the egg. Hence, a high variability in the survival of the larva should be expected because it is the mother that determines the available energy for each offspring when she assigns reserves to the egg. In turn, the available energy for a female fly to allocate at the time of oviposition also depends on many factors, such as how much energy she acquired during her larval stage, the intraspecific competition suffered in the case of superparasitism and the energy acquired by feeding as an adult. If the host-seeking larvae could allocate resource to locomotion or maintenance depending on the internal state, an extended survival could increase the chances of larvae encountering a host. An extended survival could be achieved by the accumulation of energetic reserves from the egg, which, in turn, depends on the investment the female has made when ovipositing. For example, when larvae of *Aleochara bilineata* Gyll (Coleoptera: Staphylinidae) are in the presence of a host, life expectancy reduces, probably as a result of an enhanced searching activity that reduces their energetic reserves (Royer *et al.*, 1999). By contrast, indicators of reduced life expectancy could modify decisions on resource allocation favouring an increase in the immediate reproductive effort (Clutton-Brock, 1984; Stearns, 1992; Strand & Casas, 2008). For example, oviposition of *Scotopteryx chenopoditata* Linnaeus (Lepidoptera: Geometridae) increases in response to cues such as injuries or the presence of predators, which are parameters associated with life expectancy (Javois & Tammaru, 2004). In summary, it appears that *M. ruficauda* does not completely follow the classification for parasitoids because, although parasitism is requisite for some of the larval stadia, the development of first and last stadia is independent on the host.

Parasitoidism in dipterans has evolved repeatedly across the whole lineage (Eggleton & Belshaw, 1993; Feener & Brown, 1997; Yeates & Greathead, 1997). Moreover, dipteran ancestors were saprophagous and most lifestyles are derived from this ancestral condition (Feener & Brown, 1997). Within the Asiloidea, the primitive condition of soil-dwelling predators gave rise to the different strategies found today. Although every family within the Asiloidea has a soil predator lifestyle, the Asilidae could be positioned somewhat in the middle of the evolutionary trend ranging from a soil predator behaviour to a parasitoid lifestyle (Gilbert & Jervis, 1998). Musso (1982) describes the larval behaviour of *Machimus rusticus* as micro-predatory, whereas larvae of *Mallophora media* are ectoparasitic. The example provided by *M. ruficauda* highlights the limited information that is available with respect to the evolution of the life strategies of this interesting group, and may help to shed light on the processes leading to parasitoidism.

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