Spatial density-dependent parasitism and specificity in the robber fly *Mallophora ruficauda* (Diptera: Asilidae)

MARCELA K. CASTELO[1] AND JUAN C. CORLEY[2]★

[1]*CONICET – Grupo de Investigación en Ecofisiología de Parasitoides (GIEP), Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Ciudad Universitaria, Pabellón II, (1428) Buenos Aires, and* [2]*CONICET – Laboratorio de Ecología de Insectos, INTA EEA Bariloche, (8400) Bariloche, Río Negro, Argentina (Email: jcorley@bariloche.inta.gov.ar)*

Abstract   The density-dependence in parasitism by the robber fly *Mallophora ruficauda* (Diptera: Asilidae) on scarab beetle larvae (Coleoptera: Scarabaeidae) populations was studied in the present research. *Mallophora ruficauda* is a pestiferous species common in the open grasslands of the Pampas region of South America. Adults are predators of insects and larvae are solitary parasitoids of third instar larvae of several species of scarab beetle (Coleoptera: Scarabaeidae). In contrast with most studied host-parasitoid interactions, host searching by *M. ruficauda* is carried out by both larvae and adults. Typically, robber fly females lay eggs on tall grasses from where larvae drop to the ground, and attack hosts which are buried in the soil. We carried out our study at two spatial scales close to 14 apiaries located in the provinces of Buenos Aires and Entre Ríos (Argentina). We found that parasitism is density-independent at the larger spatial scale and inversely density-dependent at the smaller one. We also found that *M. ruficauda* selects *Cyclocephala signaticollis* among several scarab beetle species. Specificity is observed both at large and small spatial scales. We discuss the implications of both host specificity and host searching behaviour on the observed parasitism patterns.

Key words: parasitoid, scarab beetle larvae, Scarabaeidae, spatial scale.

INTRODUCTION

For host-parasitoid systems, spatial density dependence – the correlation between parasitoid attacks and host density on a patch – and host specificity are both central issues of several ecological studies (Hassell 1985; Walde & Murdoch 1988). Past work has shown that spatial density-dependent parasitism plays a role on population persistence (Murdoch 1970; May *et al.* 1981; Murdoch *et al.* 1984, 1985, 1992, 2005; Hassell 1986; Bernstein 1987; Hassell & May 1988; Walde & Murdoch 1988; Murdoch & Stewart-Oaten 1989; Godfray & Pacala 1992; Murdoch & Briggs 1996; Teder *et al.* 2000). In turn, it is well known that through its influence on the functional response of parasitoids and on density dependence, host specificity may also affect population stability (Liljesthröm & Bernstein 1990; Hassell 2000). Host specificity may have negative effects on the persistence of the systems, because a specialist parasitoid might produce instability on the population of his hosts out of the built-in time-lags. In this sense, in a system with two or more host species with niche overlap, a switching generalist

could make a positive contribution towards stability, allowing species coexistence (Teramoto *et al.* 1979; Hassell 2000).

Spatial density dependence in parasitism may be either direct or inverse. Direct density dependence occurs when parasitoids are able to respond to differences in host density among patches, producing aggregation of parasitoids to higher host densities, with the subsequent increase in parasitism with density. Inverse density dependence appears when parasitism decreases with an increase in host abundance. This may occur through a lack of aggregation to high host density patches due to a 'spreading the risk' behaviour, or else, it may occur despite aggregation to high-host-density patches as a consequence of the constant rate of patch leaving by parasitoids to avoid self-superparasitism, a decelerating functional response caused by behaviours such as handling time or group defences, limitations imposed by egg availability, or interference among parasitoids (Heads & Lawton 1983; Walde & Murdoch 1988; Strong *et al.* 1990; Ives 1992; Taylor 1993; Rosenheim & Mangel 1994; Hunter 2000).

Spatial direct density dependence has been earmarked as one of several attributes contributing to the stability of host-parasitoid systems where parasitoids

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have relatively short handling times (Hassell 2000). This feature is commonly observed when the searching parasitoids spend much time, and therefore tend to aggregate, in patches of high host density. However, spatial direct density dependence on parasitism has only been found in approximately a quarter of studies reported (Stiling 1987).

One of the main issues behind this apparent mismatch is that detecting density dependence patterns is affected by several ecological factors. In a leading paper, Heads and Lawton (1983) noted the importance of spatial scale in this process (see also Stiling 1987; Walde & Murdoch 1988; Bernstein *et al.* 1991; De Roos *et al.* 1991; Hassell *et al.* 1991; Langton *et al.* 2002) and it is now known that, at larger spatial scales, direct density dependence is more frequently found than inverse density dependence probably because parasitoids are more sensitive to different host densities at larger scales (Heads & Lawton 1983; Walde & Murdoch 1988; Mohd Norowi *et al.* 2000). At smaller spatial scales, in turn, individual decisions of parasitoids during host searching can produce inverse density dependent patterns, which are frequently linked to a patchy habitat structure (Bernstein *et al.* 1991; but see Walde & Murdoch 1988). Habitat structure can alter insect movement rates and the probability that patches will be discovered and therefore is also likely to contribute to heterogeneity in parasitism (Sheehan & Shelton 1989; Cronin & Strong 1999).

Another well-known aspect affecting density dependence studies is host selection by parasitoids (Huffaker & Messenger 1976). Whereas direct density dependence is more commonly observed in monophagous parasitoids than can quickly respond to density changes of a single host species, polyphagous species respond better to changes in the general density of several host species, which in general does not result in a positive density-dependent pattern for the species involved (Huffaker *et al.* 1971; Stiling 1985; Hassell 2000). However, host switching leads to type III functional responses, which through an increase in searching efficiency of parasitoids, or a decrease in handling times, can generate density dependent patterns at low host densities. The increase in host density leads to a more than linear increase in parasitism rates, enhancing in turn, the conditions for coexistence even if hosts show complete niche overlap (Hassell 1980, 2000; Begon *et al.* 2006).

In line with this, both density dependence and specificity are features that are related to success in biological control programs of pests that use parasitoids (Mills 1997). Highly specific parasitoids will reduce the risks related with their introduction, arising by attacks on non-target species. In turn, an efficient parasitoid should act in a direct density dependent manner on host mortality factors (Huffaker *et al.* 1971; van der Bosch & Messenger 1973; Batra 1982;

Turchin 1995). This is because density dependence in parasitism implies higher host mortality at higher host densities (Stiling 1988).

For host-parasitoid systems, most spatial density dependence studies have focused on species where egg laying occurs directly in or on some developmental stage of its host (Stiling 1987; Walde & Murdoch 1988). However, a few parasitoid species lay eggs away from their hosts (Godfray 1994). While in the former, parasitoid host foraging and handling is carried out by adult females, in the latter species host searching may be shared between females and immature stages.

Host searching that is shared by adults and larvae can influence the general observed density-dependence patterns. This is because mechanisms acting during host searching at each stage imply parasitoid activities at different spatial scales. For instance, host habitat location and oviposition site selection may be carried out by highly mobile adult females in large habitats, whereas host searching patterns and host attacks that are carried out by larvae, are limited to smaller area or microhabitat (Feener & Brown 1997).

In the present paper we present the results of our studies on the spatial distribution and density dependence of parasitism by *Mallophora ruficauda* Wiedemann (Diptera: Asilidae) on its hosts, several species of scarab beetles (Coleoptera: Scarabaeidae) at two spatial scales.

Mallophora ruficauda females lay eggs in clusters on tall vegetation but parasitize hosts that are species that live buried in the soil. Thus, host searching is shared by adults and immature stages, with a limited ability to disperse and locate hosts in both adults and larvae. So, we predict that parasitism by *M. ruficauda* will be described best by an inverse density-dependent pattern at all spatial scales. This is because adults forage at large spatial scales, laying eggs in tall substrates. Female robber flies must search for plants in the grasslands and are thus limited by habitat structure. In addition, they lack the ability of close host detection and direct host attacks; features that entail inefficiencies in the parasitism processes. The female oviposition strategy produces effects on larval dispersal by means of inaccurate larvae spreading according to host position, leading to low parasitism when host abundance is high. Instead, when host abundance is low, most dispersed larvae fall in empty microhabitat sites and die without finding hosts (Castelo *et al.* 2006). In turn, parasitoid larvae passively fall to the ground at random from oviposition sites and forage for hosts in the soil within a limited range. When several individuals fall in the same microhabitat site, interference among parasitoids could occur when host availability is low, leading to superparasitism. In contrast, when host availability is high, limitations imposed by parasitoid availability, can result in low parasitism rates. Both these processes, superparasitism and para-

sitoid limitation, may imply inefficiencies that are traduced into inverse density dependence patterns of parasitism. This pattern may also arise from the fact that hosts are aggregated in the soil, and so host-parasitoid encounters result in a random pattern. The fact that *M. ruficauda* selects previously parasitised hosts, suggests also that spatial and temporal limitations influence searching for more appropriated hosts (Castelo 2003).

Applied significance to our study is given because robber flies may be able to decrease scarab beetle larvae populations, acting as efficient bio-control agents of grassland pests (Wei *et al.* 1995). Understanding parasitism patterns in the field may enhance our abilities to predict the consequences of parasitism of *M. ruficauda* on scarab beetle larvae populations. This information is relevant to the future management of robber fly populations, as they are in turn, a pest of beekeeping activities.

METHODS

Study system

Mallophora ruficauda is a pestiferous robber fly common in the open grasslands of the Pampas region of Argentina. Adults are predators of insects and larvae are solitary parasitoids of the third instar of scarab beetle larvae of several species (Coleoptera, Scarabaeidae) (Copello 1922; Castelo & Capurro 2000; Castelo & Corley 2004; Castelo & Lazzari 2004). This robber fly is the most notorious pest for the well-developed beekeeping industry of Argentina, affecting honey production by predating on worker honeybees of *Apis mellifera* L. (Hymenoptera: Apidae) during their foraging activities (Rabinovich & Corley 1997; Castelo 2003).

Mated *M. ruficauda* females deposit eggs in clusters that are placed away from the host on elevated places, typically tall grasses, or artificial supports higher than 1.25 m, in areas close to bee hives (Copello 1922; Castelo & Corley 2004). Emerging robber fly larvae are wind dispersed from the point of origin, drop to the soil from the oviposition site, and bury themselves to search for hosts (Castelo & Lazzari 2004; Castelo *et al.* 2006; Crespo & Castelo 2008). It has been established that the selection of oviposition height by the *M.*

ruficauda female contributes to larval dispersal and as a result, the parasitism success is maximal when egg-clutches are placed on substrates between 1.25 and 1.50 m in height (Castelo *et al.* 2006). Females begin oviposition in December and after 7 days parasitoid larva hatch from the eggs. Hosts are phytophagous larvae that live underground and produce damage to roots of several plants (Remedi de Gavotto 1964; Álvarez Castillo *et al.* 1993; Carmona *et al.* 1994; López *et al.* 1994; Potter 1998). Larvae of *M. ruficauda* are solitary parasitoids that feed on and finally kill their hosts when they are ready to pupate.

Field sampling methods

Field studies of host abundance and parasitism were carried out in four geographical localities of the Pampas region of Argentina: Luján (Buenos Aires, 34°34′S, 59°06′W), Pigüé (Buenos Aires, 37°37′S, 62°24′W), Victoria (Entre Ríos, 32°37′S, 60°10′W) and Mercedes (Buenos Aires, 34°40′S, 59°26′W). Sampling was done during June to July for the years 1997 to 2000. These localities are set within the major beekeeping region of Argentina, where adult robber flies feed mainly on honeybees.

Host sampling was carried out considering spatial scale (Guppy & Harcourt 1973). Distributed within the study area, a total 14 apiaries, where robber fly activity had been observed during the previous summer were sampled. Some apiaries were sampled repeatedly in different years ('sites' – the combination of apiary/year – see Table 1). In each site, we set three grids distributed through three plots with different agricultural or cattle breeding management practices ('sub-site'). Grids were placed next to wire fences and each one consisted of 50 samples taken every 2.5 m (in a parallel sense) and every 5 m (in a perpendicular sense) to the location of the apiary (10×5 respectively, Fig. 1). The individual sample (150 per site or 50 per sub-site) consisted of the extraction of a soil block of 0.35 m side and 0.30 m depth with a shovel (volume: 36.8 L; surface area: 0.12 m^2). All scarab beetle larvae were collected from each sample by digging the soil, and they were identified to the species level in the laboratory using the key of Alvarado (1980). A binocular lens was also used to register the number of larvae of *M. ruficauda* per beetle larva, which were attached externally to the host cuticle.

Table 1. Sites, apiaries, year and localities where parasites were found, during the 4 years of field work to determine the parasitism pattern of parasitoids

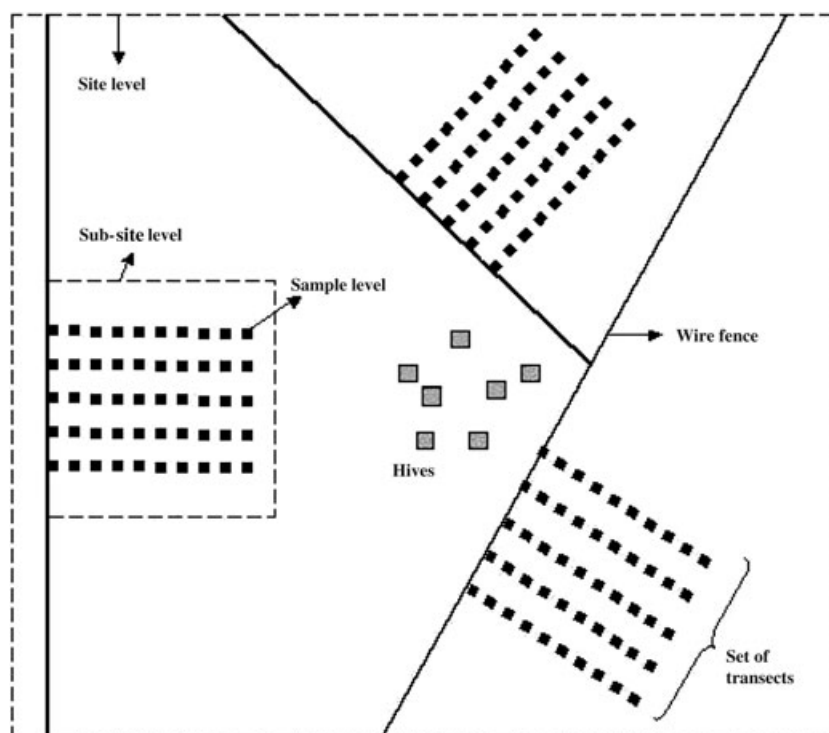| Sites	1, 2, 3, 4	5	6	7, 8, 9	10	11, 12	13, 14, 15, 16	17, 18, 19, 20, 21
Apiary	1, 2, 3, 4	5	6	1, 2, 4	5	8, 9	10, 11, 12, 13	10, 11, 12, 13, 14
Locality	Pigüé	Luján	Victoria	Pigüé	Luján	Victoria	Mercedes	Mercedes
Year	1997			1998			1999	2000

Fig. 1. Representation of how sampling of hosts and parasitoids was conducted.

Specificity analysis

Host specificity was determined both at large and small scales. However, we used different methods to assess host selectivity at each scale, according to the biology of each stage of *M. ruficauda*.

To determine host specificity in *M. ruficauda* at field scale, where the behaviour of the female has the main influence, we used the Proportional Similarity Index (*PSI*) (Feinsinger *et al.* 1981). This index provides a reliable measure of how resources are used by a population according to availability (in our case the relationship between parasitism on scarab beetle larvae and its abundance in the soil) that is, a niche width measure that analyzes the relationship between the distribution of frequencies of used resources by individuals of the population and the distribution of frequencies of available resources in the environment. The expression of the index as follows: $PSI = 1 - 0.5 \Sigma \mid pi - qi \mid$, where pi is the proportion of resource items in state i out of all items used by the population, and qi is the proportion of i items in the resource base available to the population. The underlying probability of the model is the multinomial distribution. Values for *PSI* range from 1.00 for the broadest possible niche (a population uses resources in proportion to their availability) to a minimum qx for the narrowest possible niche, where x is the rarest resource state in the resource base, when $px = 1.0$ (a population is special-

ized exclusively on the rarest resource state and consequently bypasses all other items). Although there are several measures of similarity, it has been shown that *PSI* provides an accurate estimation of selectivity in the use of resources and with powerful statistical significance, determined by the actual area of intersection between two frequency distributions. In other words, this test shows whether the distribution pi of frequencies (proportion of parasitized scarab beetle larvae of each species) is unequal to qi (proportion of scarab beetle larvae of each species founded in the field), and in this way detects specificity (Feinsinger *et al.* 1981). To establish significance levels associated with *PSI* the 95% confidence intervals were calculated using a bootstrapping procedure. We resampled data 1000 times, under the null hypothesis that each host individual has the same probability of being parasitized. Individuals that could not be determined to a specific level, due to mortality and/or decomposition of the insect bodies, were excluded from the analysis (2%).

Also, host specificity was analyzed at a sample level, in order to understand whether the larvae actively select a host once they have landed and buried themselves in the soil. We analysed specificity using an epidemiological test for prevalence, given that host seeking behaviour occurs at this spatial scale (0.20–0.30 m in diameter approximately; Castelo & Lazzari 2004). We did so by analyzing the relationship between risk exposure and its effects (Rosner 1995), in samples

with two hosts of different species, where one of them was parasitized. We carried out two evaluations of specificity, where we took the presence of individuals of 'another host species' in a sample as the exposure risk variable (y species), and parasitism on *Cyclocephala signaticollis* as the effect variable (x species), and *vice versa*. In this analysis, special emphasis was placed on *C. signaticollis* versus 'another species', because there were previous observations about the preference of *M. ruficauda* towards *C. signaticollis* (Remedi de Gavotto 1964; Crouzel 1965; Dennis & Knutson 1988; Castelo & Capurro 2000; Castelo 2003). In both cases, we compared parasitism frequencies of an individual (of x *species*) in the presence (or absence) of an individual of another species (y), in the same sample. The analysis procedure was as follows: (i) we built a contingence table with data at the sampling unit level for both studied cases; (ii) from this table we estimated relative risk of parasitism (RR) for each case using conditional probabilities, where $RR = (Sp.\ x$ parasitized/$Sp.\ y$ present)/$(Sp.\ x$ parasitized/$Sp.\ y$ not present); and (iii) we built the corresponding 95% confidence intervals (CI) for RR; and (iv) The CI was considered significant if the value one was not included in the interval, and in this way, we confirm the presence of host selectivity.

Density dependence analysis

We considered in the analysis only sites where parasitized scarab beetle larvae were found ($n = 18$). We considered two spatial scales as follows: (i) site level (large scale, apiaries), where data from 150 sampling units were pooled, ($n = 18$); and (ii) sub-site level (small scale, field lots), where data from 50 sampling units of each grid were pooled ($n = 54$) (Fig. 1). Parasitism proportions at each level were calculated as the ratio between the number of parasitized hosts and the total number of hosts found. We chose these levels of analysis because we believe they truly reflect host searching behaviour by *M. ruficauda*. While females may move among apiaries (site level) and lots (sub-site level), larvae do so only within small soil portions in the lots (Castelo & Lazzari 2004). We checked for spatial autocorrelation in scarab beetle abundance by calculating Moran's I for all plots in a given locality, irrespective of the year they were sampled. This analysis allows the identification of underlying spatial structure between samples (Rangel *et al.* 2006).

To examine the occurrence of density dependence in parasitism, we used a linear regression model, using the natural logarithm of both total host abundance and parasitized host abundance in the studied unit. To avoid overestimating the proportion of hosts bearing no parasitoids, those sites with 0% parasitism were excluded from analysis, assuming that parasitoid

larvae may not have arrived to the soil in these places or adult parasitoids did not oviposit in these specific places the previous summer. We carried out sampling in four different years because robber flies move freely and frequently among localities as a consequence of the host population dynamics and food availability. We noticed that scarab beetle larva abundance is very variable among years due to different causes (crop management, field conditions, parasitism outcome itself), so in the next period, the presence of *M. ruficauda* and the levels of parasitism for a given site are variable too. Also, it is frequent that beekeepers change hive emplacement from one field to another each year for productivity reasons. Given this scenario, it becomes necessary to redefine sampling places each year. Another reason to do surveys in several years was to get an appropriate number of sites to carry out valid statistical analysis. We note that is not easy to find high numbers of apiaries with the presence of *M. ruficauda* during any given sampling period.

To detect density dependence, Dennis and Taper (1994) have suggested the use of a resampling method, solving the excessive Type I error produced by the regression method. We studied density dependence by using the methodology proposed by Capurro *et al.* (1997). First we carried out a lineal regression between the variables mentioned, and then we controlled the regression significance as follows: (i) we calculated 'Jack-knife' estimations for the regression slope as its variance; (ii) we built the 95% CI for the slope using Z distribution (Efron 1982; Caswell 1989); and (iii) the regression was considered statistically significant if the slope equal to zero is not included within the confidence interval.

In addition, with the purpose of confirming the occurrence or lack of density dependence, we tested the presence of a relationship between the total number of scarab beetle larvae and the number of parasitized scarab beetle larva at both spatial scales by means of a Generalized Linear Model, applying a logistic regression and including sites with 0% parasitism (binomial GLM, GenStat 10.2; Hardy 2002).

RESULTS

We observed hosts parasitized by *M. ruficauda* in 18 out of 21 sampled sites. Data of total and parasitized host abundance are summarized in Table 2. For the larger part of the soil samples, no hosts were found ($n = 1716$).

Specificity analysis

We collected 2515 scarab beetle larvae in all sites, of which 2454 could be identified at species level, and

Table 2.   Number of individual of host species collected at apiary scale by year (no. parasitized hosts shown in parentheses)

Species	2000	1999	1998	1997	Total
CS	218 (56)	136 (30)	156 (31)	435 (63)	945 (180)
CM	52 (9)	15 (1)	21 (1)	94 (3)	182 (14)
CP	36 (0)	19 (2)	16 (0)	10 (1)	81 (3)
DA	21 (1)	4 (0)	31 (0)	37 (1)	93 (2)
PB	467 (4)	301 (1)	126 (0)	111 (2)	1005 (7)
BS	0	0	2 (0)	0	2 (0)
AT	65 (1)	51 (0)	3 (0)	6 (0)	125 (1)
AV	6 (1)	3 (0)	9 (0)	0	18 (1)
HB	0	0 (0)	0	3 (0)	3 (0)
Total	865 (72)	529 (34)	364 (32)	696 (69)	2454 (208)

AT, *Anomala testaceipennis*; AV, *Archophileurus vervex*; BS, *Bothynus striatellus*; CM, *Cyclocephala modesta*; CP, *Cyclocephala putrida*; CS, *Cyclocephala signaticollis*; DA, *Diloboderus abderus*; HB, *Heterogeniates bonariensis*; PB, *Philochloenia bonariensis*.
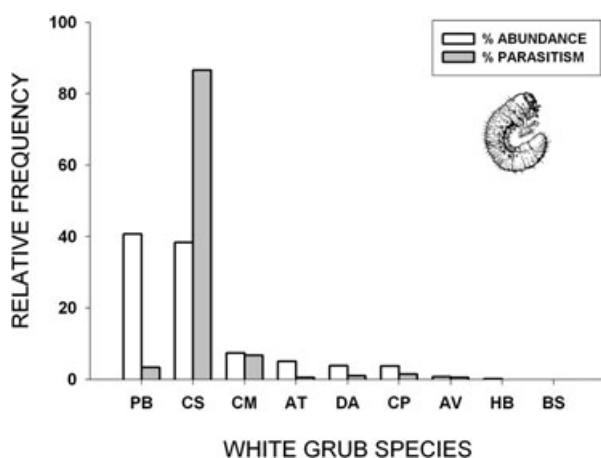

Fig. 2.   Global frequency of scarab beetle larva species detected in the analyzed apiaries (filled bars) and parasitized species of beetle larvae by *Mallophora ruficauda* according to the environmental availability (white bars) from 18 sites (see text for details). AT, *Anomala testaceipennis*; AV, *Archophileurus vervex*; BS, *Bothynus striatellus*; CM, *Cyclocephala modesta*; CP, *Cyclocephala putrida*; CS, *Cyclocephala signaticollis*; DA, *Diloboderus abderus*; HB, *Heterogeniates bonariensis*; PB, *Philochloenia bonariensis*.

208 were parasitized by *M. ruficauda*. Nine scarab beetle larvae species were found in the samples: *Cyclocephala signaticollis* Burmeister (CS), *Cyclocephala modesta* Burmeister (CM), *Cyclocephala putrida* Burmeister (CP), *Diloboderus abderus* Sturm (DA), *Philochloenia bonariensis* Bruch (PB), *Archophileurus vervex* Burmeister (AV), *Anomala testaceipennis* Blanchard (AT), *Bothynus striatellus* Fairmaire (BS), and *Heterogeniates bonariensis* Ohaus (HB). Among these, only the last two species were not attacked by *M. ruficauda* (Fig. 2). All species except AV, BS and HB were found every year in the studied places. The only difference among years is the proportion of individuals of each scarab beetle larva species (Table 2). We assume that the cause of this variation in abundance

relates to crop rotation carried out every 6 months in this region. Proportional Similarity Index (*PSI*) was on average 0.52 (mean value of all sites calculated by year, simulated *PSI* (mean) = 0.946, lower 95% CI = 0.908, $P < 0.001$), which shows that *M. ruficauda* uses only almost half of the available scarab beetle larva items, which correspond to three species, *C. signaticollis*, *C. modesta* and *C. putrida*, and where preference of *M. ruficauda* towards *C. signaticollis* larvae is highlighted (Fig. 2, Table 3). When we studied host specificity at the micro spatial scale, we detected that an individual of *C. signaticollis* had a probability of parasitation of 0.27 when another host species was present in the same sample and 0.31 when it was alone, the difference not being significant (the *RR* of parasitization was 0.87, CI = [0.61; 1.24]). For any nother species, we found that the probability dropped to 0.03 when *C. signaticollis* was present in the same sample. When *C. signaticollis* was absent, the probability increased slightly but significantly 0.05 (the *RR* of parasitation was 0.62, CI = [0.40; 0.95]).

Density dependence analysis

The general percentage of parasitism at the Site level was 10.2% (SD = 6.16; range 1.46–21.86%; $n = 18$). We did not detect a significant linear relationship between host abundance (ln) and percent parasitism (ln) at Site scale ($r^2 = 0.07$; $P = 0.2936$; $b = -0.29$; $n = 18$; GLM: $t = -1.74$, $P = 0.082$; Fig. 3a). At the small spatial scale, a significant negative linear relationship between host abundance and percent parasitism was obtained for sub-site level ($r^2 = 0.26$; $P < 0.0006$; $b = -0.48$; $n = 42$; GLM: $t = -2.06$, $P = 0.039$; Fig. 3b; confidence intervals for Jack-knife estimations calculated for the regression slope did not include zero, CI = [−0.757; −0.223]).

There was no spatial autocorrelation of scarab beetle densities at both site and sub-site spatial scales, for all

Table 3.   Calculation of the Proportional Similarity Index (*PSI*) to determine host specificity by *Mallophora ruficauda*

Resource state	(Abundance WG) No. available items	q_i	(Parasitized WG) Total used	p_i	p_i-q_i	$|p_i$-$q_i|$
CS	945	0.3851	180	0.8654	0.4803	0.4803
CM	182	0.0742	14	0.0673	−0.0069	0.0069
CP	81	0.0330	3	0.0144	−0.0186	0.0186
DA	93	0.0379	2	0.0096	−0.0283	0.0283
PB	1005	0.4095	7	0.0337	−0.3759	0.3759
BS	2	0.0008	0	0	−0.0008	0.0008
AT	125	0.0509	1	0.0048	−0.0461	0.0461
AV	18	0.0073	1	0.0048	−0.0025	0.0025
HB	3	0.0012	0	0	−0.0012	0.0012
TOTAL	2454		208			0.9606

PSI = 0.52. *P* < 0.001. AT, *Anomala testaceipennis*; AV, *Archophileurus vervex*; BS, *Bothynus striatellus*; CM, *Cyclocephala modesta*; CP, *Cyclocephala putrida*; CS, *Cyclocephala signaticollis*; DA, *Diloboderus abderus*; HB, *Heterogeniates bonariensis*; PB, *Philochloenia bonariensis*.
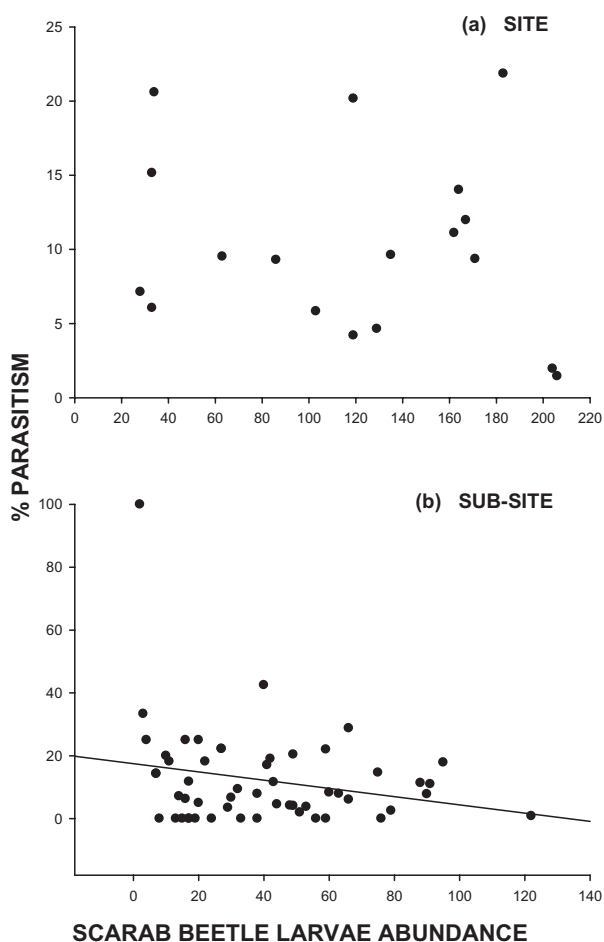

Fig. 3.   Relationship between host abundance in the soil and percentage of parasitized hosts by *Mallophora ruficauda*, for all sites studied during 1997–2000 in four localities. (a) Site level (apiary). (b) Sub-site level (lot). We detected density independence at site level (a) and inverse density dependence at sub-site level (b).

years studied (Moran's I, 500 iterations; site level all years: $n = 14$, I = −0.340 to 0.156, *P* = 0.21 to 0.87; sub-site level 1997: $n = 15$, I = −0.255 to 0.062, *P* = 0.30 to 0.68, 1998: $n = 15$, I = −0.173 to 0.198, *P* = 0.07 to 0.86, 1999: $n = 12$, I = −0.488 to 0.465, *P* = 0.07 to 0.80) except for year 2000 ($n = 12$, I = −1.178 to 0.307, *P* = 0.06 to 0.776). This is probably best explained by the very different management of the fields rather than by their geographical separation.

DISCUSSION

We studied spatial density dependent parasitism by the robber fly *M. ruficauda* on scarab beetle larvae. The observed patterns were density-independent at a large spatial scale and inversely density-dependent at the smaller scale. Our study also shows that *M. ruficauda* attacks mainly *C. signaticollis* among seven potential host species. The patterns we observed in this host-parasitoid system where host searching is a two-step process are essentially those previously noted for other host-parasitoid interactions.

The spatial scale of analysis is important because parasitism patterns can vary with it, and these variations may be accounted by different behavioural processes dominating each scale (Heads & Lawton 1983; Walde & Murdoch 1988; Rothman & Darling 1990; Bernstein *et al.* 1991; De Roos *et al.* 1991; Hassell *et al.* 1991; Mohd Norowi *et al.* 2000). For instance host searching, host attack and host localization mechanisms by females are very relevant in this sense.

At larger spatial scales, generalist adult parasitoids can produce a density independent pattern of parasitism, because cycles of host and parasitoid abundance may not be necessarily synchronized. But, also at large spatial scales, density dependence can occur, when parasitism rates are very different among sites as a

consequence of also variable local abiotic factors. As host density can be variable, foraging parasitoids may not be able to distinguish areas with high host density (Heads & Lawton 1983). For the *M. ruficauda*–scarab beetle system, we observed density-independent parasitism patterns at the larger spatial scale studied. We speculate this may be a consequence of adult females missing host-rich patches. Oviposition behaviour in this species suggests that females select places that maximize larval dispersal, probably because hosts are buried in the soil and are widely distributed in the habitat (Castelo 2003; Castelo & Corley 2004). The contribution of adults to host searching is probably minimal, except for the contribution that mothers may make to increased larval dispersal (see Castelo *et al.* 2006).

At smaller spatial scales, most parasitoids are able to detect hosts within a limited area surrounding each host. This is accomplished through signals referred to the host's environment (Tumlinson *et al.* 1993; Godfray 1994). An outcome of parasitoids spatial detection threshold coupled with a limited number of parasitoids in relation to hosts, leads to an inverse density-dependent pattern of parasitism. Host attacks by *M. ruficauda*, rather than being carried out by mobile adults as in most parasitoid species, are carried out within the soil by larvae, and are thus limited to a very small area (Castelo & Lazzari 2004; Crespo & Castelo 2008). The fact that *M. ruficauda* accepts previously parasitized hosts also suggests that spatial and temporal limitations influence the search for more appropriated hosts or host patches (Castelo 2003).

Host selectivity, another important attribute of density-dependence studies in host-parasitoid systems, typically produces strong direct density dependence (Huffaker *et al.* 1971; Huffaker & Messenger 1976; Stiling 1985). A purely specialist parasitoid will be best described by a very marked direct density dependent parasitism pattern at large spatial scales, as a consequence of the coincidence of abundance fluctuations between host and parasitoid populations. Although we detected host specificity by *M. ruficauda*, we noted the absence of direct density dependence at the studied spatial scales. This fact may be related to the shared host searching strategy between females and larvae, where females search for oviposition sites and larvae search actively for hosts, at a spatial scale that is determined by the site of landing.

In a previous similar study, Castelo and Capurro (2000) found for this system, direct-density dependence at site level. They argued then, that direct density-dependence was a consequence of the fact that female robber flies would lay eggs in environments with high host density, hypothesising that the adult female may have some skill to qualify environments according to host density. Our present results, which include a larger dataset, suggest that parasitism at the site level is density-independent. This sheds light on the importance of adequate sampling in detecting true density-dependence, but also, suggests that in line with recent work (Castelo & Corley 2004; Castelo *et al.* 2006), adult females may not be host searching, but instead laying eggs in a manner that enhances larval dispersal.

An additional finding of our work suggests that *M. ruficauda* may be of limited importance to scarab beetle larva population regulation. While inverse density dependence is detected at the smallest scale, at the largest scale the observed pattern of parasitism is density-independent. These results can imply that control on *M. ruficauda* populations, related to their impact on beekeeping (e.g. egg cluster removal, adult mortality), may bring about minimal consequences on the population dynamics of another pest, as scarab beetle may be for lawns and grasslands.

This contribution is one of a series of studies focused on understanding the behavioural mechanisms and ecological consequences of host searching by the robber fly *M. ruficauda*. In recent work, we have looked at how this fly seeks hosts through chemical cues during its larval stages (Castelo & Lazzari 2004; Crespo & Castelo 2008), whether adult females choose oviposition sites (Castelo & Corley 2004), and how parasitism could be maximized by appropriate oviposition and consequent larvae dispersal (Castelo *et al.* 2006).

Our main conclusion is that despite marked differences between the host searching strategy displayed by some dipteran parasitoids, at different spatial scales, and others whose females lay eggs directly on their hosts as many Hymenoptera do, the general patterns of density dependence in parasitism are similar. Parasitoid host searching behaviour either carried out by adults only or displayed as a shared strategy between adults and immature stages with limited mobility, may both lead to inverse density dependence at small spatial scales and density independence at larger scales. As expected, environmental heterogeneity also plays an important role in larvae host localization and in the systems regulation. Because, the broad knowledge of the conventional host-parasitoid systems may not be directly applicable to robber flies, further specific studies are required to fully understand the population dynamics of this unique system.

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