Behavioural Response in an Asilid Fly: Influence of Ecological and Environmental Factors on Spatial Density Dependence

MARCELA K. CASTELO (mcastelo@ege.fcen.uba.ar)
Universidad de Buenos Aires. Ciudad Universitaria

JOSÉ E. CRESPO
Universidad de Buenos Aires. Ciudad Universitaria  https://orcid.org/0000-0002-0074-6924

Research Article

Keywords: parasitoid, scarab-beetle larvae, Scarabaeidae, spatial scale, parasitism.

DOI: https://doi.org/10.21203/rs.3.rs-529383/v1

License: ☒ ☀ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Behavioural response of a parasitoid shows the effect on host parasitism patterns at a given host distribution resulting in an increase or decrease of parasitism intensity according to local host densities. This relationship could be proportional, positive, or negative, as a consequence of foraging of parasitoids searching for hosts. *Mallophora ruficauda* is a fly parasitoid of *Cyclocephala signaticollis* scarab beetle larvae and a predator of honeybees. Females search and place egg-clusters overground in open grasslands near beehives. Larvae actively searching for host underground following chemical cues arising from the host itself. The parasitism pattern is a result of this complex host-searching strategy which is shared between both stages of the fly. In this work we carried out a study at four spatial scales in apiaries located in the Pampas region of Argentina. We found that parasitism is inverse density-dependent at high female activity and direct density-dependent at low female activity at the larger spatial scale. We found a direct density dependent pattern associated to substrate height at intermediate spatial scale that is lost when the habitat has abundant oviposition substrates. Conversely, parasitism is inversely density-dependent at both smaller spatial scales, associated to oviposition substrate distance and saturation of healthy host by larvae attacking. Additionally, *M. ruficauda* does not select the oviposition substrates according to the abundance of *Cyclocephala signaticollis* inhabiting underground. This work shows the importance of a proper scale for analysis of factors that influence population dynamics and how environmental characteristics mould parasitism patterns in this dipteran parasitoid.

Introduction

The term behavioural response of a parasitoid has been defined by Hassell (Hassell 1966) as a behaviour response to the distribution of hosts that, being distributed unevenly, results in an increase or decrease of parasitism intensity according to local host densities. Hence, studying different dynamics of host-parasitoid systems has been subject of great interest (Walde and Murdoch 1988, Gunton and Pöyry 2016). In particular, mechanisms leading to stability are useful not only from a theoretical view but also in applied science given the importance of parasitoids as biological control agents (Waage and Hassell 1982, Bernstein 1987, Fernández-arhex and Corley 2003, Jervis 2005). Spatial density dependence has been rendered as an important factor leading to stability as well as host specificity through its influence on the functional response of parasitoids and on density dependence (Bernstein 1987, Hassell 2000).

Spatial density dependence in parasitism is the outcome of parasitoids responding to differences in host density among patches leading to changes in the intensity in parasitism (Walde and Murdoch 1988). Past work has shown that spatial density-dependent parasitism plays a role on population persistence and stability of the host-parasitoid systems (May et al. 1981, Murdoch et al. 1984, 1985, 1992, 2005, Hassell and May 1988, Murdoch and Stewart-Oaten 1989, Godfray and Pacala 1992, Murdoch and Briggs 1996, Teder et al. 2000). When parasitoids aggregate as a response to high host densities, with a consequent increase in parasitism percentage then direct density dependence occurs. On the contrary, if parasitism percentage decreases with increasing host abundance, inverse density dependence occurs. Many studies have dealt with establishing the relation between parasitism and host abundance and...
examples of direct, inverse or independent density dependence have been found (Stiling 1987, Walde and Murdoch 1988, Gunton and Pöyry 2016). Nonetheless an important remark should be made. In a leading paper, Heads and Lawton (1983) noted the importance of spatial scale in this process. They showed that expected patterns of prey mortality imposed by a population of natural enemies aggregating in response to victim densities can vary from exponential curves when the sample area is smaller than a “patch” through transient intermediate relationships to an increasing relationship when the “patch” size is recognised by the natural enemy (Heads and Lawton 1983).

In a more recent update, Gunton and Pöyry (2016) introduced the “scale-specific foraging hypothesis” implying that the nature of observed correlations between local host densities and parasitism rates is the result of methodological artefacts of the observational scales used. They propose that parasitoids discriminate among host patches according to their density at a “foraging grain size” that normally creates a positive density-parasitism relationship. But, as they point out, this relationship will be detected as long as the size of the study units within which densities are calculated is comparable to the foraging grain size.

Other important factors that have been studied that influence spatial density dependence can be summarized in host characteristics. For instance, host distribution can influence profoundly parasitoid’s response size where optimal strategies can vary for highly aggregated or randomly distributed hosts (Walde and Murdoch 1988). Within hosts characteristics, exotic hosts and large bodied parasitoids seem to be associated to negative parasitism responses (Gunton and Pöyry 2016). Although there are many theoretical approaches and experimental studies trying to shed light on the effects and mechanisms of spatial density dependence on population dynamics, there are still needs for multi-scale studies. Even more if we consider that most of the studies (~ 85%) refer to hymenopteran parasitoids that differ greatly in the searching and oviposition strategies with other parasitoids (e.g. dipterans) (Godfray 1994, Feener Jr and Brown 1997, Gunton and Pöyry 2016).

In this work we analyse spatial density dependence parasitism in a system of a dipteran parasitoid and its coleopteran host. Mallophora rucauda Wiedemann (Diptera: Asilidae) 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 koinobiont ectoparasitoids of the second and third instar of scarab beetle larvae Cyclocephala signaticollis Burmeister (Coleoptera, Scarabaeidae) (Castelo and Capurro 2000, Castelo and Corley 2010, Crespo and Castelo 2010). Mated M. rucauda 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 (Castelo and Corley 2004, 2010). Emerging robber fly larvae are wind dispersed, drop to the soil from the oviposition site, and bury themselves searching for hosts (Castelo and Lazzari 2004, Castelo et al. 2006, Crespo and Castelo 2008). It has been established that the selection of oviposition height by the M. rucauda female contributes to larval dispersal and, as a result, the parasitism success is maximal when egg-clusters are placed on substrates between 1.25 and 1.50 m in height (Castelo et al. 2006). Hosts are phytophagous larvae that live underground and produce damage to roots of several plants (Potter 1998, Carrasco et al. 2011).
Regarding host location, this parasitoid has a split strategy in which both female and larvae are involved. Firstly, female places its eggs on tall substrate as mentioned before. After being wind dispersed, first larval instar moults to the next instar and then active host searching occurs. In order to find its host, *M. ruficauda* larva orientates to its host through detection of cues produced in the host’s posterior intestine (Castelo and Lazzari 2004, Crespo and Castelo 2008, Groba and Castelo 2012). Larvae of *M. ruficauda* can discriminate hosts from different instars, parasitism status and species (Crespo and Castelo 2008, Crespo et al. 2015, Martínez et al. 2017). Given the split strategy in this system involved in host location, second instar larva can be claimed to be the ecological equivalents of female in hymenopteran parasitoids. Hence, it is justified to ask if spatial density dependence of parasitism exists in this system.

Previous studies on this host-parasitoid system have shown contradictory results when analysing spatial density dependence at a large spatial scale, but both were consistent finding an inverse density dependence pattern at the smaller spatial scale (Castelo and Capurro 2000, Castelo and Corley 2010). One of them found evidence of direct density dependence at a spatial scale compatible to adult patches (Castelo and Capurro 2000) while the other study, which included increased sample sites, found no relation at the larger spatial scale (Castelo and Corley 2010). Both studies were performed including the potential host species that make up the community of rizophagous Scarabaeidae larvae in the study area, introducing a potential confounding factor because *M. ruficauda* has a marked preference for *C. signaticollis* larvae (Castelo and Crespo 2012). This work is motivated in the clarification of the results found in previous studies with the addition firstly of more sample sites and secondly much more information on host specificity in this species and other ecological features regarding oviposition height and distance to the hosts, substrate availability, and activity of females. So, the goal of this work is to show the results of our studies on the spatial density dependence of parasitism by *M. ruficauda* on its preferred host, *C. signaticollis* at different spatial scales. We also show how the inclusion of information from habitat use by females and host use by larvae combine to properly determine the spatial density patterns at different spatial scales.

**Material And Methods**

**Field sampling methods**

Field studies of host abundance and parasitism were carried out in six geographical localities of the Pampas region of Argentina: Luján, Mercedes, Moreno, Pigüé, Pilar and Victoria. Sampling was done during June to August between 1997 and 2006. These localities are within the major beekeeping region of Argentina, where adult robber flies feed mainly on honeybees (Figure 1).

The study was carried out in 17 fields with apiaries where robber fly activity was registered in the previous summer. Some fields were sampled repeatedly in different years, so the combination apiary/year was defined as the scale “site” (see Table 1). In each site, three plots with different agricultural or cattle breeding management practices and vegetation (from now on “sub-site” scale) were sampled. On each sub-site a grid was placed next to the wire fence. Grids were made up of 10 lines of 5 samples parallel to
the wire fence ("Line level"). Samples were taken every 2.5 m within each line. Lines were placed every 5 m covering a total of 50 m. Each sample (small scale) consisted of the extraction of a soil block of 0.35 m side by 0.30 m depth obtained with a shovel (volume: 36.8 L; surface area: 0.12 m$^2$). In sum, samples were grouped in lines (5 samples per line) with 10 lines per sub-site obtaining 50 samples in total. The largest scale, “site”, consisted of 3 sub-sites, hence 150 samples (Figure 2).

From each block of soil, all scarab beetle larvae were collected by manually breaking the soil and identified to the species level in the laboratory with a dichotomic key (Alvarado 1980). A stereomicroscope was used to register the number of larvae of *M. ruficauda* attached to the host cuticle. Only *C. signaticollis* larvae were counted as host abundance since it is the preferred host for *M. ruficauda* (Crespo and Castelo 2010, Barrantes and Castelo 2014).

Scales were chosen because we believe they represent the true complexity in this system. As mentioned before, *M. ruficauda* adults belong to a genus of robust dipterans that feed on other flying insects like honey bees (Bromley 1930, 1946, Cole and Pritchard 1964, Corley and Rabinovich 1997). Asilids have an important flying capacity, near 1-2 km, easily covering a site area (Kanmiya 2002, Londt 2020). Once the female places its eggs on the substrate, larvae will be wind dispersed, so the sub-site together with the line levels capture mainly the influence of wind on larval dispersal and a possible effect of distance to the oviposition site but no further influence of females. Finally, the sample scale captures host-searching performed by the larva itself after dropping and burying into the soil.

**Density dependence analysis**

We considered in the analysis only sites where parasitized scarab beetle larvae were found ($n = 25$). We carried out sampling in different years because parasitoids move freely and frequently among localities as a consequence of the host population dynamics and food availability. *Cyclocephala signaticollis* larvae abundance might be very variable among years due to different causes (crop management, field conditions, parasitism outcome itself) introducing variability in the presence of *M. ruficauda* and parasitism levels at a given site. Due to this scenario, it was necessary to redefine sampling places every year.

For each of the scales analysed, (i) site (apiaries); (ii) sub-sites (field lots); (iii) lines (transects within field lots) and (iv) samples (unitary block of soil), proportion of parasitized hosts was calculated as the ratio between the number of parasitized *C. signaticollis* and the total number of *C. signaticollis* found. To avoid overestimating the proportion of hosts bearing no parasitoids, those sites with 0% parasitism were excluded from analysis, assuming that parasitoid larvae may have not arrived to the soil in these places or adult parasitoids did not oviposit in those specific places the previous summer.

We analysed the proportion of parasitized *C. signaticollis* through generalized linear models. For each scale we generated a model that included different predictors informative of that scale (site, sub-site, line and sample models). All models were generated with a Binomial distribution and a logit link function. After modelling a full model, model selection was performed. For every model, the effect of dropping an
interaction or a predictor variable (with \(\text{drop1}\) function) was evaluated through the Likelihood ratio test and the AIC value. After obtaining the minimal model, significant terms were evaluated with the \text{anova} function. Finally, interaction plots of the estimated marginal means were done to explore the relation between the predictors and the response variable.

For the site model host abundance and amount of egg-clusters in place were used as predictor variables. Host abundance was included as a discrete predictor variable while amount of egg-clusters was included as a categorical predictor variable with two levels (low and high). Egg-clusters was included as a predictor variable since the abundance of \(M. \text{ruficauda}\) cannot be directly calculated.

The sub-site full model was constructed with host abundance as discrete predictor variable and vegetation height as a categorical predictor variable (low or high). Vegetation height is an indicator of oviposition substrate availability for \(M. \text{ruficauda}\) (Castelo and Corley 2004). In fields with low vegetation height, only wire fences are available for oviposition while many other oviposition substrates (\(e.g.\) tall grasses, stems, sticks) are also available in fields with high vegetation. If only wire fences are available for oviposition, egg-cluster aggregation could occur as a result of availability of oviposition substrates. However, egg-cluster aggregation could still occur in vegetation if females are attracted to odours from damaged plants, hosts or other egg-clusters favouring oviposition in specific substrates. Attraction to damaged plants has already been discarded since oviposition on dry plants and wire fences are frequent (Castelo et al. 2006). In order to discard female attraction to host odours, we studied if female \(M. \text{ruficauda}\) places more egg-clusters on plants and wire fences associated with hosts at a small scale. For this, we registered the position of between 28 and 35 plants or wire fences with egg-clusters, in six apiaries with proven presence of \(M. \text{ruficauda}\) in the previous summer. From each plant and wire fence, the total number of egg-clusters placed by \(M. \text{ruficauda}\) females was registered. Wire fences were 2m portions of longitudinal wire randomly chosen. Plants geographic positions were registered because the following step of the study was performed during autumn and many plants were gone by then. Hence, in autumn, soil samples were obtained using the same technique as previously described. Soil samples with previous plant positions were taken using the geographic position as the centre. For samples from wire fences, soil samples were obtained from the midpoint of the wire longitude. Soil samples were analysed to quantify the number of larvae of \(C. \text{signaticollis}\) present. With data from number of egg-clusters and number of hosts, a model was constructed with the former as the response variable and the latter as a discrete numerical explanatory variable. The influence of the number of \(C. \text{signaticollis}\) hosts on the number of egg-clusters was evaluated with a glm with a Gamma distribution and log link function with the ID of each site as a random factor.

Next, line model included the same predictor variables as sub-site model (host abundance and vegetation height) and distance to the wire fence as another discrete predictor variable. This variable accounted for any distance effect that could be introduced in fields with low vegetation height.

Finally, sample model included the same predictor variables as the line model (host abundance, vegetation height and distance to the wire fence). We assumed that dependency on sites and sub-sites
would not introduce a notorious effect on the results since females are not able to place two egg-bouts on a single day (M. Castelo, personal observation). Given the fact that at maximum only 3 sub-sites per site could be included as random effects, they were not included as random variables because at least five replicates is suggested to estimate variance.

All the statistical analyses were performed using R version 3.6.3 “Holding the Windsock” (R Core Team 2020). Models were performed with the function `glmmTMB` from the glmmTMB package (Brooks et al. 2017). Interaction plots of the estimated margin means were performed with `emmin` function of the library emmeans (Lenth et al. 2020). Plots were done with the library ggplot2 (Wickham et al. 2020).

Data available from the Dryad Digital Repository: Castelo and Crespo 2020.

**Results**

We observed *C. signaticollis* hosts parasitized by *M. rucauda* in 18 out of 37 sampled sites. Data of total and parasitized host abundance are summarized in Tables 1 and 2. For the larger part of the soil samples, no hosts were found (*n* = 4959). We collected 887 *C. signaticollis* of which 172 were parasitized by *M. rucauda* (~19.39% of parasitism).

**Density dependence analysis**

The four models showed information that can be separated in three (site model, subsite-model and line-sample models together). For every model we found support for either direct or inverse density dependence.

For the site model we found that the relationship between the proportion of parasitism and the number of hosts depended on the estimated abundance of *M. rucauda* through the abundance of egg-clusters found. In sites where estimated abundance of *M. rucauda* was high, we found inverse density dependence. On the contrary, in sites where the estimated abundance of *M. rucauda* was low, a direct density dependence relation was found (Fig. 3A, Table 2).

For the sub-site model, we found that the relationship between the proportion of parasitism and the number of *C. signaticollis* hosts depended on the vegetation height. In sub-sites where vegetation height was high, we found density independence. On the contrary, in sites where the vegetation height was low (only wire fences as oviposition substrates), a direct density dependence relation was found (Fig. 3B, Table 3). We also found no relation between the abundance of *C. signaticollis* hosts and the number of egg-clusters in substrates indicating that oviposition is not related to plants and wire fences associated with hosts at a small scale (Chisq$_3$ = 1.936, *P* = 0.586).

For the line and sample models, we found an inverse density dependence between the proportion of parasitism and the number of hosts (Figs. 3C and 3D, Tables 4 and 5). In both models no influence of distance to the wire fence or vegetation height was found.
Discussion

In this work we show the results of our studies on spatial density dependent parasitism by the robber fly *M. ruficauda* on *C. signaticollis* scarab beetle larvae at different spatial scales. We found two different scenarios, *i.e.* at large spatial scales either direct density dependence or inverse density dependence was found. At smaller spatial scales only inverse density dependence was found. The patterns we found in this system might be related to the two-step stages involved in host searching. In this kind of parasitoids (dipteran, coleopteran, lepidopteran, neuropteran, strepsipteran and trichopteran; Mills 2009) where the female cannot access directly to the concealed host, females place their eggs close to hosts and it is the larva that must locate the host (Godfray 1994, Feener Jr and Brown 1997).

The spatial scale of analysis is central in determining parasitism patterns since the variations may be accounted by different behavioural processes dominating each scale (Heads and Lawton 1983, Walde and Murdoch 1988). There are studies showing a switch in parasitism patterns as a result of changes in host searching behaviour when parasitoids use different cues at different spatial scales (Jarošík and Lapchin 2013), or as a result of demographic processes like aggregation of parasitoids on natural vegetation near crops or promotion of high female fecundities (Segoli 2016, Morgan et al. 2017).

At large spatial scales, generalist adult parasitoids can produce a density independent pattern of parasitism when parasitism rates vary among sites as a consequence of variable local abiotic factors. As host density can be variable, foraging parasitoids may not be able to distinguish areas with high host density. However, when the sample area is bigger than the effective patch, then density dependence patterns might be lost (Heads and Lawton 1983). In *M. ruficauda* we observed inverse density dependent parasitism when the abundance of adults was high and direct density dependence pattern was observed when the adult abundance was low. This result lends support to both previous studies that found contradictory results (Castelo and Capurro 2000, Castelo and Corley 2010). Although previous studies found support for opposed density dependent patterns, both studies included as potential hosts, larvae of other species than *C. signaticollis* because it was thought that *M. ruficauda* could develop on several Scarabaeidae host species. However, it has been already shown that *M. ruficauda* can only develop to imago on *C. signaticollis* as its host albeit positive orientation toward other *Cyclocephala* species occur (Barrantes and Castelo 2014). Also, previous studies did not include information regarding vegetation or wire fences height, distance to the wire fences or adult activity. This information, as shown through our analysis, proved relevant for *M. ruficauda* given its split host location strategy.

Another important difference that might influence the results found at the site scale could be related to the size of the site area that included fields with many different characteristics. The Pampas region where *M. ruficauda* is typically found has been highly modified for livestock and agricultural purposes. Site scale therefore includes fields with crops and livestock culture that introduces high variability. The abundance of hosts in fields with livestock culture or soybean crops is much lower than abundance in cereal or potato crops and soy or sunflower cultivars. Another possible explanation for the difference found with previous studies at site scale might be that Asilidae adults are territorial (Onsager and Rees
1985, Lavigne and Bullington 2001, Weber and Lavigne 2004, Haab et al. 2019). A particular type of competition occurs in this species since oviposition often occurs on substrates where a previous egg-cluster had been placed by another female (M. Castelo, personal observation). This particular behaviour could introduce competition where high amount of egg-clusters produce an inverse density pattern since not all larvae from egg-clusters will be able to reach a host or several larvae could reach the same host incurring in inefficient superparasitism.

The results so far could indicate that female M. ruficauda could lay eggs in environments with high host density and may have some skill to qualify environments according to host abundance supporting conclusions drawn in previous studies (Castelo and Capurro 2000). However, Castelo and Corley’s (2010) study included a larger dataset and found a density-independent parasitism at the site scale. This highlights on the importance of adequate sampling (replication and spatial scale) in detecting true density-dependence.

We then analysed the sub-site scale that matches the surface of fields with an homogenous type of land use. At this spatial scale, we found both direct and independent density patterns depending on the vegetation height of the field was low and high respectively. This apparent difference in the results could be explained by the oviposition behaviour that M. ruficauda has (Castelo and Corley 2004, Castelo et al. 2006). Females select the higher available substrates maximizing larval dispersal and minimizing superparasitism (Castelo et al. 2006). In fields with low vegetation height the only suitable oviposition substrates were the wire fences (~ 1 m), while on fields with high vegetation heights many other oviposition substrates were available. Given the sampling grids that were settled starting at the wire fence, fields with lower vegetation might be revealing the true process in this system. Since larva are dispersed by the wind, the distance travelled by the larva will depend on environmental conditions like wind velocity and height of the oviposition site. The parasitism pattern found at this scale will have contributions of the females that decided where to place the egg-clusters and will be the result of larval dispersion. Hence, this spatial scale could be truly representing the patch size recognized by M. ruficauda showing a direct density dependence. On the contrary, in fields with high vegetation, density independence was found. This result should not be surprising since the availability of many oviposition sites could generate a confounding effect tarnishing the pattern.

At smaller spatial scales we found the same result as in previous studies, i.e. inverse density dependence. These results may be explained by the fact that larvae of M. ruficauda are wind dispersed and cannot actively select landing place. Once larvae land to the soil they bury themselves and, after moulting, start searching for their hosts orienting to host chemical cues (Castelo and Lazzari 2004, Crespo and Castelo 2008). This type of anemophilic dispersal imposes dispersion limits into this system generating a top limit in the amount of parasitoid larvae that can land in any specific spot. Given also that C. signaticollis hosts do not distribute randomly but tend to aggregate in space and are also attracted by the same chemical cues used by M. ruficauda, the resulting spatial pattern is inversely dependent. The spatial distribution of small particles wind dispersed in a heterogeneous habitat has been successfully used in modelling the spatial distribution of individuals from different species in forests (Shen et al. 2009). In this
work, the authors showed that both dispersal limitation and habitat heterogeneity were important factors in determining spatial distribution of propagules. Also, soil factors could affect the risk of parasitism because of differences in host accessibility, as the depth to which hosts bury underground in a patch (Okuyama 2019). The idea that larval dispersion in *M. ruficauda* can be modelled as a seed dispersion phenomenon has already been explored with results that explained the oviposition strategy in this species (Castelo et al. 2006). Hence, an inverse density dependence at small spatial scales could be indicating that dispersal limitation in an heterogeneous habitat is the driving processes.

The dispersal of larvae is highly dependent on the female oviposition site selection. Hence, it is important to understand if females place more egg-clusters in substrates associated with more hosts underground that could be releasing attractive chemical cues. However, we found no relation between the amount of egg-clusters in a specific plant and the abundance of *C. signaticollis* at the plant scale. This result reinforces the idea that *M. ruficauda* does not use host related cues associated to plants or wire fences. In fact, *M. ruficauda* females would be using substrates mainly based on its height which should minimize superparasitism and increase singly parasitism as it has been already proposed (Castelo et al. 2006). However, we have frequently registered egg-clusters clumped on the same plant in the field (*M. Castelo*, personal observation). It is unknown if egg-cluster aggregation poses some adaptive advantage for females during oviposition site seeking or if female use some other characteristic of substrates indicative of good habitat quality. These characteristics could be other relevant factors related to their life cycle like host densities at different spatial scales, prey abundance or even human related activities like the agricultural management.

An important conclusion of our work could indicate that *M. ruficauda* actually could be considered of importance to control *C. signaticollis* population opposed to suggestions previously made considering several species of Scarabaeidae larvae (Castelo and Corley 2010). These results reinstates the discussion that actions against *M. ruficauda* to lessen their impact on beekeeping (*e.g.* egg cluster removal, adult mortality), may bring not negligible consequences on the population dynamics of *C. signaticollis*.

Our main conclusion is that in parasitoid-host systems with complex host searching strategies also show different parasitism patterns at different spatial scales. In fact, according to the ‘scale-foraging-hypothesis’ of Gunton and Pöyry (2016), the nature of the observed correlations is strongly affected by the observational spatial scale and supports the idea that parasitoids discriminate among host patches according to their density at a ‘foraging grain size’. However, when studying parasitism patterns and the influence of density dependence it is important to analyse potential effects of ecological factors such as dispersion limitation or habitat heterogeneity that have important roles in shaping complex population dynamics.

**Declarations**

**ACKNOWLEDGMENTS**
The authors are indebted to Ángel Capurro and Juan Corley for their invaluables ideas and fruitful discussions.

**Funding** This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (grant number PIP 0368CO) and Universidad de Buenos Aires (grant numbers UBACyT 0059BA, 0019BA).

**Conflicts of interest/Competing interests** Authors declare no conflict of interest.

**Ethics approval** All applicable institutional and/or national guidelines for the care and use of animals were followed.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Availability of data and material** The data was deposited in [repository name] under the reference number [identifier number].

**Code availability** Not applicable.

**Authors' contributions** MKC conceived and designed the experiments. MKC and JEC performed the experiments. JEC analyzed the data. MKC and JEC wrote the manuscript.

**References**

1. Alvarado L (1980) Sistematica Bionomia Coleopteros Estados Inmaduros Viven Suelo. Universidad Nacional de La Plata

2. Barrantes ME, Castelo MK (2014) Host specificity in the host-seeking larva of the dipteran parasitoid *Mallophora ruficuda* and the influence of age on parasitism decisions. Bulletin of Entomological Research 104:295–306. https://doi.org/10.1017/S0007485314000029

3. Bernstein C (1987) On Assessing the Role of Spatially-Heterogeneous Density-Independent Host Mortality on the Stability of Host-Parasitoid Systems. Oikos 49:236–239. https://doi.org/10.2307/3566033

4. Bromley SW (1930) Bee-Killing Robber Flies. Journal of the New York Entomological Society 38:159–176

5. Brooks ME, Kristensen K, Benthem KJ van, et al (2017) glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. The R Journal 9:378–400

6. Carrasco N, Báez A, Belmonte ML (2011) Trigo. Manual de campo. In: INTA. https://inta.gob.ar/documentos/trigo-manual-de-campo. Accessed 25 Jul 2020

7. Castelo M karina, Corley JC (2004) Oviposition Behavior in the Robber Fly *Mallophora ruficuda* (Diptera: Asilidae). esaa 97:1050–1054. https://doi.org/10.1603/0013-8746(2004)097[1050:OBITRF]2.0.CO;2
8. Castelo MK, Capurro Á (2000) Especificidad y denso-dependencia inversa en parasitoides con oviposición fuera del hospedador: el caso de Mallophora ruficauda (Diptera: Asilidae) en la Pampa Argentina. Ecología Austral 10:89–101

9. Castelo MK, Corley JC (2010) Spatial density-dependent parasitism and specificity in the robber fly Mallophora ruficauda (Diptera: Asilidae). Austral Ecology 35:72–81. https://doi.org/10.1111/j.1442-9993.2009.02013.x

10. Castelo MK, Crespo JE (2012) Incidence of Non-Immunological Defenses of Soil White Grubs on Parasitism Success of Mallophora ruficauda Larva (Diptera: Asilidae). Insects 3:692–708. https://doi.org/10.3390/insects3030692

11. Castelo MK, Lazzari CR (2004a) Host-seeking behavior in larvae of the robber fly Mallophora ruficauda (Diptera: Asilidae). Journal of Insect Physiology 50:331–336. https://doi.org/10.1016/j.jinsphys.2004.02.002

12. Castelo MK, Lazzari CR (2004b) Host-seeking behavior in larvae of the robber fly Mallophora ruficauda (Diptera: Asilidae). Journal of Insect Physiology 50:331–336. https://doi.org/10.1016/j.jinsphys.2004.02.002

13. Castelo MK, Ney-Nifile M, Corley JC, Bernstein C (2006) Oviposition height increases parasitism success by the robber fly Mallophora ruficauda (Diptera: Asilidae). Behavioral Ecology and Sociobiology 61:231–243. https://doi.org/10.1007/s00265-006-0254-5

14. Corley J, Rabinovich M (1997) An important new predator of honey bees-the robber fly Mallophora ruficauda Wiedemann (Diptera, Asilidae) in Argentina. American Bee Journal 137:303–306

15. Crespo JE, Castelo MK (2010) Life-history traits in a parasitoid dipteran species with free-living and obligate parasitic immature stages. Physiological Entomology 35:160–167. https://doi.org/10.1111/j.1365-3032.2010.00727.x

16. Crespo JE, Castelo MK (2008a) The ontogeny of host-seeking behaviour in a parasitoid dipteran. Journal of Insect Physiology 54:842–847. https://doi.org/10.1016/j.jinsphys.2008.03.002

17. Crespo JE, Castelo MK (2008b) The ontogeny of host-seeking behaviour in a parasitoid dipteran. Journal of Insect Physiology 54:842–847. https://doi.org/10.1016/j.jinsphys.2008.03.002

18. Crespo JE, Martínez GA, Castelo MK (2015) Exposure to competitors influences parasitism decisions in ectoparasitoid fly larvae. Animal Behaviour 100:38–43. https://doi.org/10.1016/j.anbehav.2014.11.005

19. Feener Jr DH, Brown BV (1997) Diptera as Parasitoids. Annual Review of Entomology 42:73–97. https://doi.org/10.1146/annurev.ento.42.1.73

20. Fernández-arhex V, Corley JC (2003) The Functional Response of Parasitoids and its Implications for Biological Control. Biocontrol Science and Technology 13:403–413. https://doi.org/10.1080/0958315031000104523

21. Godfray HC, Pacala SW (1992) Aggregation and the population dynamics of parasitoids and predators. Am Nat 140:30–40. https://doi.org/10.1086/285401

22. Godfray HCJ (1994) Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press
23. Groba H f., Castelo M k. (2012) Chemical interaction between the larva of a dipteran parasitoid and its coleopteran host: A case of exploitation of the communication system during the searching behaviour? Bulletin of Entomological Research 102:315–323. https://doi.org/10.1017/S0007485311000691

24. Gunton RM, Pöyry J (2016) Scale-specific spatial density dependence in parasitoids: a multi-factor meta-analysis. Functional Ecology 30:1501–1510. https://doi.org/10.1111/1365-2435.12627

25. Haab KA, McKnight TA, McKnight KB (2019) Phenology and Ethology of Adult *Lasiopogon slossonae* Cole and Wilcox Robber Flies (Diptera: Asilidae) in a New York Riparian Habitat. went 121:594–615. https://doi.org/10.4289/0013-8797.121.4.594

26. Hassell MP (1966) Evaluation of Parasite or Predator Responses. Journal of Animal Ecology 35:65–75. https://doi.org/10.2307/2690

27. Hassell MP (2000) The Spatial and Temporal Dynamics of Host-Parasitoid Interactions, Edición: 1. Oxford University Press, Oxford; New York

28. Hassell MP, May RM (1988) Spatial heterogeneity and the dynamics of parasitoid-host systems. Annales Zoologici Fennici 25:55–61

29. Heads PA, Lawton JH (1983) Studies on the Natural Enemy Complex of the Holly Leaf-Miner: The Effects of Scale on the Detection of Aggregative Responses and the Implications for Biological Control. Oikos 40:267–276. https://doi.org/10.2307/3544591

30. Jarošík V, Lapchin L (2013) An experimental investigation of patterns of parasitism at three spatial scales in an aphid-parasitoid system (Hymenoptera: Aphidiidae). EJE 98:295–299. https://doi.org/10.14411/eje.2001.050

31. Jervis MA (ed) (2005) Insects as Natural Enemies: A Practical Perspective. Springer Netherlands

32. Kanmiya K (2002) Flight properties of orthorrhaphous Brachycera flies in tethered flight performance (Insecta: Diptera). Medical Entomology and Zoology 53:109–120. https://doi.org/10.7601/mez.53.109_2

33. Lenth R, Singmann H, Love J, et al (2020) emmeans: Estimated Marginal Means, aka Least-Squares Means. Version 1.4.8URL https://CRAN.R-project.org/package=emmeans

34. Londt JGH (2020) A survey of grassland Asilidae (Diptera) at Jacana Eco Estate, Hilton, South Africa. African Invertebrates 61(1):29–48. https://doi.org/10.3897/AfrInvertebr.61.50895

35. Martínez GA, Castelo MK, Crespo JE (2017) Behavioural plasticity induced by intraspecific competition in host orientation in a parasitoid. Ecological Entomology 42:484–491. https://doi.org/10.1111/een.12407

36. May RM, Hassell MP, Anderson RM, Tonkyn DW (1981) Density Dependence in Host-Parasitoid Models. Journal of Animal Ecology 50:855–865. https://doi.org/10.2307/4142

37. Mills N (2009) Chapter 190 - Parasitoids. In: Resh VH, Cardé RT (eds) Encyclopedia of Insects (Second Edition). Academic Press, San Diego, pp 748–751
38. Morgan WH, Thébault E, Seymour CL, van Veen FJF (2017) Density dependence and environmental factors affect population stability of an agricultural pest and its specialist parasitoid. Biocontrol 62:175–184. https://doi.org/10.1007/s10526-016-9777-5

39. Murdoch W, Briggs CJ, Swarbrick S (2005) Host Suppression and Stability in a Parasitoid-Host System: Experimental Demonstration. Science 309:610–613. https://doi.org/10.1126/science.1114426

40. Murdoch WW, Briggs CJ (1996) Theory for Biological Control: Recent Developments. Ecology 77:2001–2013. https://doi.org/10.2307/2265696

41. Murdoch WW, Briggs CJ, Nisbet RM, Stewart-Oaten A (1992) Aggregation and Stability in Metapopulation Models. The American Naturalist 140:41–58. https://doi.org/10.1086/285402

42. Murdoch WW, Chesson J, Chesson PL (1985) Biological Control in Theory and Practice. The American Naturalist 125:344–366

43. Murdoch WW, Reeve JD, Huffaker CB, Kennett CE (1984) Biological Control of Olive Scale and Its Relevance to Ecological Theory. The American Naturalist 123:371–392. https://doi.org/10.1086/284210

44. Murdoch WW, Stewart-Oaten A (1989) Aggregation by Parasitoids and Predators: Effects on Equilibrium and Stability. The American Naturalist 134:288–310. https://doi.org/10.1086/284981

45. Okuyama T (2019) Density-dependent distribution of parasitism risk among underground hosts. Bulletin of Entomological Research 109:528–533. https://doi.org/10.1017/S0007485318000871

46. Potter DA (1998) Destructive Turfgrass Insects: Biology, Diagnosis, and Control, Edición: 1. Wiley, Chelsea, Mich.

47. Segoli M (2016) Effects of habitat type and spatial scale on density dependent parasitism in *Anagrus* parasitoids of leafhopper eggs. Biological Control 92:139–144. https://doi.org/10.1016/j.biocontrol.2015.10.011

48. Shen G, Yu M, Hu X-S, et al (2009) Species–area relationships explained by the joint effects of dispersal limitation and habitat heterogeneity. Ecology 90:3033–3041. https://doi.org/10.1890/08-1646.1

49. Stiling PD (1987) The Frequency of Density Dependence in Insect Host-Parasitoid Systems. Ecology 68:844–856. https://doi.org/10.2307/1938356

50. Teder T, Tanhuanpää M, Ruohomäki K, et al (2000) Temporal and spatial variation of larval parasitism in non-outbreaking populations of a folivorous moth. Oecologia 123:516–524. https://doi.org/10.1007/s004420000346

51. Waage JK, Hassell MP (1982) Parasitoids as biological control agents – a fundamental approach. Parasitology 84:241–268. https://doi.org/10.1017/S003118200005366X

52. Walde SJ, Murdoch WW (1988) Spatial Density Dependence in Parasitoids. Annual Review of Entomology 33:441–466. https://doi.org/10.1146/annurev.en.33.010188.002301
Tables

Table 1. Sites, apiaries, year and localities where sites were found, during the 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    |     |        |                 |                   |

Table 2. Estimated regression parameters, standard errors, z-values and P-values for the site model. abundance_CS is the abundance of *C. signaticollis* hosts. Low-egg-clusters are the sites where the estimated abundance of *M. ruficauda* was low.

|                          | Estimate | S.E. | z val. | p    |
|--------------------------|----------|------|--------|------|
| (Intercept)              | -0.14    | 0.55 | -0.25  | 0.79 |
| abundance_CS             | -0.04    | 0.02 | -2.86  | <0.01|
| low-egg-clusters         | -1.82    | 0.68 | -2.69  | 0.01 |
| abundance_CS:low-egg-clusters | 0.06  | 0.02 | 3.52   | <0.01|

Table 3. Estimated regression parameters, standard errors, z-values and P-values for the sub-site model. abundance_CS is the abundance of *C. signaticollis* hosts. vegHeightLow are the sub-sites with low vegetation (only wire fences as oviposition substrates).
Table 4. Estimated regression parameters, standard errors, $z$-values and $P$-values for the line model. abundance_CS is the abundance of *C. signaticollis* hosts.

|                  | Estimate | S.E. | $z$ val. | $p$  |
|------------------|----------|------|----------|------|
| (Intercept)      | -0.52    | 0.55 | -0.96    | 0.34 |
| abundance_CS     | -0.03    | 0.04 | -0.64    | 0.52 |
| vegHeightLow     | -1.00    | 0.79 | -1.27    | 0.20 |
| abundance_CS:vegHeightLow | 0.11 | 0.06 | 1.76 | 0.08 |

Table 5. Estimated regression parameters, standard errors, $z$-values and $P$-values for the sub-site model. abundance_CS is the abundance of *C. signaticollis* hosts.

|                  | Estimate | S.E. | $z$ val. | $p$  |
|------------------|----------|------|----------|------|
| (Intercept)      | 0.99     | 0.21 | 4.75     | <0.01|
| abundance_CS     | -0.21    | 0.04 | -5.76    | <0.01|

---

**Figures**
Figure 1

Map of the sampling sites.

Figure 2

Representation of how sampling of hosts and parasitoids was conducted.
Figure 3

Fit of the four models. A) site model. B) sub-site model. C) line model. D) sample model.