

# A hierarchical multi-scale analysis of the spatial relationship between parasitism and host density in urban habitats

MARÍA SILVINA FENOGLIO,<sup>1\*</sup>  VICTORIA WERENKRAUT,<sup>2</sup>  
JUAN MANUEL MORALES<sup>2</sup> AND ADRIANA SALVO<sup>1</sup>

<sup>1</sup>Facultad de Ciencias Exactas Físicas y Naturales, Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET and Centro de Investigaciones Entomológicas de Córdoba (CIEC), Universidad Nacional de Córdoba, Av. Vélez Sarsfield 1611, X5016GCA Córdoba (Email: msfenoglio@unc.edu.ar), and <sup>2</sup>Laboratorio Ecotono, INIBIOMA, Universidad Nacional del Comahue-CONICET, S. C. de Bariloche, Río Negro, Argentina

**Abstract** Studies on spatial density dependence in parasitism have paid scarce attention to how changes in host density at different hierarchical scales could influence parasitism in an herbivore at a particular scale. Here, we evaluated if rates of parasitism per leaf (by the whole parasitic complex and by dominant species) of the specialist leaf miner *Liriomyza commelinae* (Diptera: Agromyzidae) respond to variations in host density at the leaf, plant patch and site levels in an urban setting. We used multi-level Bayesian models that incorporate the spatial hierarchy occurring in this system, as well as habitat factors previously found to have an effect on the *L. commelinae* parasitoid community in an urban context (patch size, patch isolation and urbanization level). According to the fitted model, overall parasitism rates decreased with increasing number of mines per leaf, being independent of host-density variations at patch and site level. Patch structure was found to have a strong effect on parasitism rates per leaf. The analysis of parasitism by parasitoid species separately showed consistent results with the response at community level. These results suggest that parasitism of the parasitoid community here studied would be sensitive to hierarchical cues related to the host at the leaf level and to the host habitat at the patch level.

**Key words:** density dependence, isolation, leaf miner, parasitoid assemblage, patch size, urbanization.

## INTRODUCTION

The relationship between mortality from parasitoids and host density in space, known as spatial density dependence in parasitism, has been a relevant issue in the ecological study of host–parasitoid interactions because of the role it plays in the persistence and stability of populations (Hassell & May 1973; May *et al.* 1981; Comins *et al.* 1992) as well as for its implications on pest control (Murdoch & Briggs 1996). Even though the importance of analysing the relationship across scales has long been recognized (Heads & Lawton 1983; Rothman & Darling 1991; Ives *et al.* 1993; Ray & Hastings 1996; Norowi *et al.* 2000; Schellhorn & Andow 2005), scarce attention has been paid to how spatial changes in host density at different hierarchical scales could influence the parasitism of an insect herbivore at a particular scale.

A hierarchical multi-scale approach may be important in studying parasitism–density relationships because (i) parasitoid females are known to search for hosts on a hierarchy of spatial scales (Völkl 1994) and (ii) herbivorous insects may often present

variations in their distribution and abundance that respond to a hierarchy of spatial scales (Walde & Murdoch 1988). The conceptual model of host finding by parasitoids (Salt 1935; Laing 1937) proposes that parasitism is part of a hierarchical process involving, from the higher to the smaller scales: host–habitat location, host location, host acceptance and host suitability. Thus, female parasitoids have to make decisions inherent to each scale, such as whether to forage for hosts or food, which cues to use, where to go and how long to stay (Vet *et al.* 2002). Moreover, in heterogeneous environments, parasitoid species have to deal with patchily distributed resources often represented by herbivorous insects that occur in hierarchical patches, consisting of leaves, plants and groups of plants.

According to this nested organization, if parasitism is evaluated on the smallest natural unit of the habitat (i.e. the leaf), a hierarchical influence of host-density variations on the probability of an herbivore to be parasitized could be expected. At a small scale, since parasitism would match host density, direct spatial density dependence is predicted due to parasitoid aggregation, in accordance with the optimal foraging theory (Charnov 1976; Cook & Hubbard 1977) and host–parasitoid models (Hassell 2000). Moreover, in

\*Corresponding author.

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parasitoid species foraging on aggregated hosts, oviposition would increase the probability of staying in a leaf patch via an incremental mechanism (van Alphen *et al.* 2003). However, classic reviews on the topic have shown that parasitism rates can also decrease or remain unchanged with host density (Lessells 1985; Stiling 1987; Walde & Murdoch 1988). Inverse density dependence in parasitism could be expected at finer scales (Norowi *et al.* 2000; Jarošík & Lapchin 2001; Castelo & Corley 2010) if mechanisms like egg limitation, prolonged handling time, 'spreading the risk' behaviour, or parasitoid interference are acting (Lessells 1985; Walde & Murdoch 1988; van Alphen & Jervis 1996; Visser *et al.* 1999). At an intermediate spatial scale, such as the plant, host density would influence the probability of an herbivore to be attacked on a leaf, determining the amount of both olfactory (Vet & Dicke 1992; Geervliet *et al.* 1998; Hilker & McNeil 2008; Girling *et al.* 2011) and visual (Casas 1988; Sugimoto *et al.* 1988; Fischer *et al.* 2001; Bora & Deka 2014) cues that parasitoid females use when selecting suitable patches for oviposition. In fact, there is evidence that parasitoids are capable of responding to differences in quality without the need to make direct contact with host patches in order to assess their profitability (Fischbein *et al.* 2012). At a large spatial scale, such as the level of groups of plants at a site, host density may be important since herbivores located on a leaf of a plant patch with moderate host abundance may be more heavily attacked if the patch occurs in the neighbourhood of other patches with high host abundance (Veldtman & McGeoch 2004). The rates of parasitoid attacks at a particular site could be affected by host density through population level responses, since parasitoid movement between sites is expected to be very low (Walde & Murdoch 1988; Ives *et al.* 1993; Philpott *et al.* 2009).

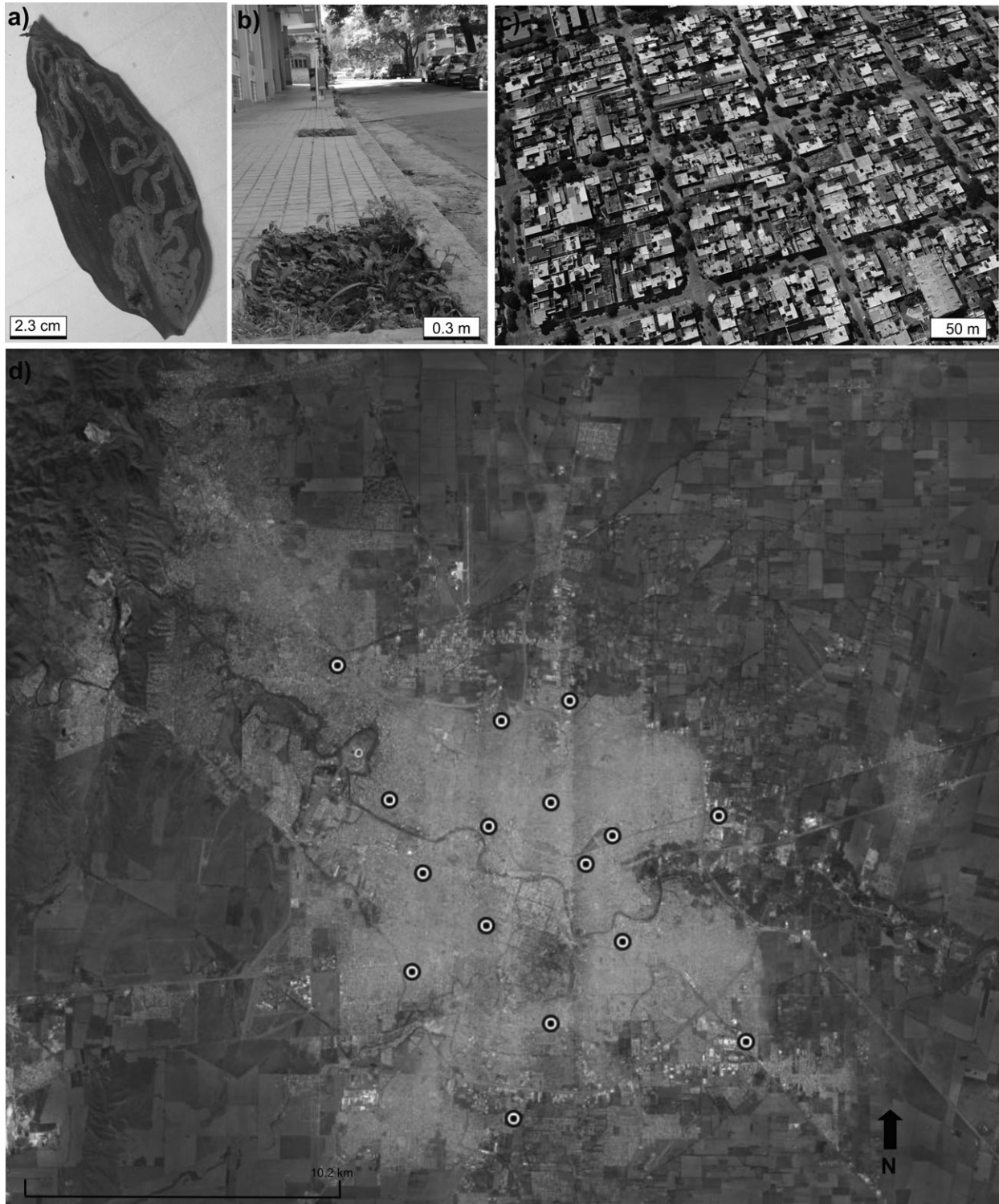
Leaf-mining insects and their parasitoid assemblages inhabiting urban landscapes are an interesting system to study parasitism–density relationships across scales for several reasons. Firstly, most of the species in this herbivore guild may perceive a single leaf as a food item but also as a feeding patch, since a leaf can often support more than one developing larva, leading to variations in host density (Hespenheide 1991). Thus, the spatial scale where leaf miners truly interact with parasitoids, from the point of view of the herbivore, is represented by the leaf. Secondly, this group of herbivores, as well as others in these habitats, can have variation in their spatial distribution due to high environmental heterogeneity (McIntyre 2000; Raupp *et al.* 2010), leading to a series of hierarchies of host spatial distribution. Thirdly, the frequent and simultaneous occurrence of multiple parasitoid species associated with leaf miners (Hawkins 1994) offers an interesting but poorly studied

aspect in the investigation of the host density–parasitism relationship (Heads & Lawton 1983; Stireman & Singer 2002; Costamagna *et al.* 2004). Fourthly, although urban land use has been shown to decouple plant–herbivore–parasitoid interactions at multiple spatial scales (Nelson & Forbes 2014), density dependence has been scarcely analysed in these habitats (but see Thorarinsson 1990). Within cities, factors other than host density, related to both the local patch and/or the surrounding landscape, could also influence parasitoids (Burkman & Gardiner 2014). Thus, a comprehensive analysis of the relationship between parasitism and density should include host and host habitat variables across scales.

The present study evaluated if parasitism rates per leaf (overall and from the dominant species) of the specialist leaf miner *Liriomyza commelinae* (Frost, 1931) (Diptera: Agromyzidae) respond to variations in host density at the leaf, plant patch and site levels in an urban setting (Fig. 1). For this purpose, we used multi-level Bayesian models that incorporate the spatial hierarchy occurring in this system as well as habitat factors previously found to have an effect on the parasitoid community of *L. commelinae* at different scales in an urban context. The Bayesian approach is convenient for modelling hierarchically structured data (Gelman & Hill 2006) since it allows accounting for scale effects in analysis and inference (McMahon & Diez 2007). Traditional analyses that compare independent studies done at different scales usually fail to account for correlations shared by scales, and fail to estimate variation at each scale given variation at the others (Underwood & Petraitis 1993).

Previous analysis of the dataset explored here showed that parasitism rates of *L. commelinae* at patch level increased in large and less isolated plant patches through enhanced parasitoid species richness (Fenoglio *et al.* 2013). Also, increasing host abundance at patch level was found to have a diluted effect on parasitism in larger patches. At the site level, changes in species composition were detected along the urbanization gradient due to differential sensitivity of parasitoids to environmental disturbance (Fenoglio *et al.* 2009). Here, we focus on host-density effects across scales on parasitism rates per leaf. The scales of analyses were not arbitrarily selected, but on the basis of the natural spatial variations of *L. commelinae* in the urban habitat. It is known that (i) *L. commelinae* density increases with urbanization degree (Fenoglio *et al.* 2009), (ii) large patches of the host plant sustain higher abundance of the leaf miner (Fenoglio *et al.* 2010) and (iii) the leaf miner has a clumped distribution at the leaf scale, independently of patch size (Fenoglio 2009; Aparicio *et al.* 2015).

A study of density dependence across several spatial scales has been previously performed for



**Fig. 1.** Hierarchical structure of the system (a) leaf of *Commelina erecta* mined by *Liriomyza commelinae* (b) plant patch of *C. erecta* (c) urban blocks representing the site level (d) location of sites ( $n = 16$ ) in the city of Córdoba (Argentina).

parasitism in *L. commelinae* (Freeman & Smith 1990). However, this is the first study that includes variation in host density at different scales in an

urban system in a single analysis that intends to explain parasitism at one particular scale. We predict that increases in host density at the leaf, patch and

site scales will positively influence the proportion of *L. commelinae* larvae parasitized on a leaf; we also expect that host density measured at the same spatial scale as parasitism will have the strongest effect.

## METHODS

### Study system

*Liriomyza commelinae* is a neotropical species that was recorded feeding on plants within the genera *Commelina* and *Tradescantia* (Commelinaceae) (Stegmaier 1966; Smith 1987), but appears to be a specialized feeder on *Commelina erecta* L. in central Argentina (Valladares 1984). This herbaceous plant species frequently appears as a component of the spontaneously growing vegetation in cities, showing a highly patchy distribution (Fenoglio *et al.* 2010). Larvae of *L. commelinae* burrow into the leaf parenchyma, forming distinct serpentine mines visible on the upper surface of leaves, within which they pupate (Smith 1987). Peak abundance occurs during the summer, particularly in February and March. This marked seasonality seems to coincide with the growing period of *C. erecta* (Sérsic *et al.* 2006).

Parasitoids are the main source of mortality of *L. commelinae*, with 25 native hymenopteran species being recorded in central Argentina causing on average 50% mortality (Fenoglio & Salvo 2009). Most of these parasitoids are solitary and polyphagous, feeding on various leaf-mining species in the study region (Salvo & Valladares 1999; Fenoglio & Salvo 2009). Whereas obligatory hyperparasitism is rare among leaf miner parasitoids, facultative hyperparasitism could be rather common (Askew & Shaw 1974), but was not quantified in this study. Without laborious and detailed dissections, rates of facultative hyperparasitism cannot be determined and thus our results reflect the net outcome of parasitism (Lewis *et al.* 2002). Successful superparasitism, the emergence of two or more parasitoids from a single host, could potentially inflate the percentage parasitism values. Given the rearing methodology employed here, it was not possible to discard its occurrence. However, successful superparasitism was reported as rare in field studies, at least for species related to those here recorded (Patel *et al.* 2003; Song *et al.* 2004; Mafi & Ohbayashi 2010).

### The data

We used a subset of the data of the tritrophic system *C. erecta*–*L. commelinae* parasitoid community previously analysed by Fenoglio *et al.* (2009, 2013). Those publications dealt with variables at patch (size, isolation, host abundance) and site (urbanization index) level respectively, but did not incorporate data at the leaf scale. *Commelina erecta* plant patches were surveyed in each site as reported in Fenoglio *et al.* (2009, 2013). All mined leaves per patch were collected and transported to the laboratory where the number of mines per leaf was recorded. Each leaf was individually placed in plastic bags and kept until emergence of flies and parasitoids. We decided to use 16 from 18 sites

originally sampled in Córdoba city since two sites had a very low number of replicates at leaf scale (<7). Plant patches ranged in size from 0.0025 to 11.6 m<sup>2</sup> (mean ± SE = 28.34 ± 3.91) and distance between patches, from 0.5 to 120 m (mean ± SE = 11.54 ± 0.81). The index used to estimate the urbanization level of each site was obtained from a multivariate analysis using the following variables: distance from the city centre, vehicular traffic, vegetation cover at the landscape level, surface area dedicated to gardens and ground temperature (see Fenoglio *et al.* 2009 for further details). The index varied from –2.94 (least urbanized) to 2.35 (most urbanized).

The response variable here evaluated was the realized parasitism (i.e. proportion of host larvae producing adult parasitoids) (Stoepler & Lill 2013), which was measured at the leaf scale as overall parasitism rate (total number of parasitoids divided by the total number of leaf miners) and parasitism rate caused by individual species (total number of parasitoids of the species *i* divided by the total number of leaf miners). The selected parasitoid species were the four most abundant: *Chrysocharis* sp. A, *C. flacilla*, *C. vonones* and *Chrysonotomyia* sp. B (all Eulophidae).

For host-density variables, we used the total number of mines as indicative of host density at the leaf scale, whereas for each plant patch and site we calculated *L. commelinae* density as the sum of the number of mines per leaf within the plant patch/site divided by the plant patch/site size. Site size was estimated as the sum of plant patch sizes within the site. The size and isolation of plant patches and the index of urbanization were included in the analyses as other predictor variables.

### Statistical analysis

We modelled the total number of parasitoids with a binomial distribution as follows:

$$y_i \sim \text{Binomial}(p_i, a_i)$$

where  $i = 1$ –3087 and indicates the leaf,  $y_i$  is the total number of parasitoids at leaf $_i$ ,  $p_i$  is the probability of parasitoid emergence at leaf $_i$ , and  $a_i$  is the total number of adults emerged from leaf $_i$  (leaf miners plus parasitoids).

To account for spatial nesting and to explore the effects of predictor variables on parasitism rates at three spatial scales (leaf, plant patch and site), we fitted a hierarchical Bayesian model where the hierarchy was modelled at intercept level. We incorporated predictor variables at the three spatial levels. The probability of parasitoid emergence ( $p_i$ ) was linked with the predictor variables via the logit function. Our model was as follows:

$$\text{logit}_{p_i} = \alpha_{0j} + \alpha_1 \text{number of mines}_i$$

where  $i = 1$ –3087 and indicates the leaf,  $j = 1$ –430 and indicates the plant patch, and  $\alpha_1$  is the regression coefficient for the effect of number of mines at the leaf scale.

At the plant patch level, the varying plant patch intercepts ( $\alpha_0$ 's) were regressed against the plant patch-scale predictors, as follows:

$$\alpha_{0j} \sim N(\beta_{0k} + \beta_1 \text{size}_j + \beta_2 \text{isolation}_j + \beta_3 \text{mine density}_j, \sigma_\alpha^2)$$

where  $N$  represents the normal distribution,  $i$  and  $j$  are the same as above,  $k = 1-16$  and indicates the site,  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  are the regression coefficients for the effects of patch size, isolation and mine density, respectively, and  $\sigma_\alpha^2$  is the within-plant patch variance.

At the site level, the varying site intercepts ( $\beta_0$ 's) were regressed against the site-scale predictors, as follows:

$$\beta_{0k} \sim N(\gamma_0 + \gamma_1 \text{size}_k + \gamma_2 \text{urbanization}_k + \gamma_3 \text{mine density}_k, \sigma_\beta^2)$$

where  $k$  is the same as above,  $\gamma_1$ ,  $\gamma_2$  and  $\gamma_3$  are the regression coefficients for the effects of site size, urbanization and mine density, respectively, and  $\sigma_\beta^2$  is the within-site variance.

We estimated model parameters with the software WinBUGS (Lunn *et al.* 2000) and the R2WinBUGS package (Sturtz *et al.* 2005) in the R statistical program (R Development Core Team, 2012). We ran five parallel chains of 1 000 000 Markov Chain Monte Carlo (MCMC) iterations; we discarded the first half as burn-in and kept 50 000 simulations. We used non-informative priors and random initial values. We assigned normal prior distributions with mean = 0 and standard deviation = 1000 to parameters  $\alpha_1$ ,  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ ,  $\gamma_0$ ,  $\gamma_1$ ,  $\gamma_2$  and  $\gamma_3$ . For variance parameters ( $\sigma_\alpha^2$  and  $\sigma_\beta^2$ ), we assigned uniform prior distributions between 0 and 100. Convergence was assessed by using the Gelman-Rubin statistic ('Rhat', Gelman *et al.* 2003) with all diagnostic values <1.1 indicating convergence (Gelman *et al.* 2003). Before the analyses, we log-transformed and standardized all predictors (mean = 0, standard deviation = 1), which allowed direct comparison of their relative explanatory power by means of their standardized coefficients (Schielzeth 2010). The full model specification is provided in Appendix S1.

We adjusted the model described above for the whole parasitoid community. In addition, with the aim of exploring whether an individual species shows the same tendency as that of community, we adjusted the same model for each of the four most abundant parasitoid species of the complex (see Results). These results should be interpreted with caution because our models do not include effects of competition or spatial or temporal segregation (Amarasekare 2000).

## RESULTS

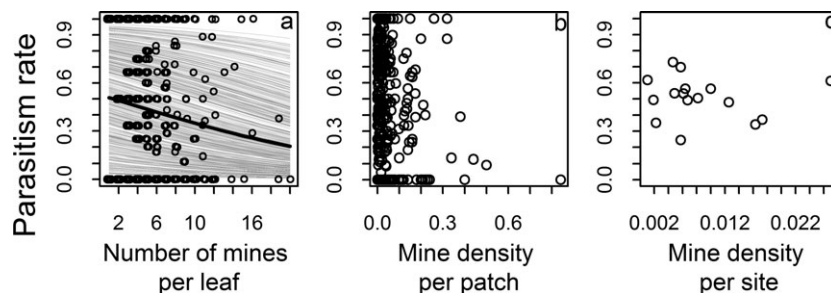
A total of 3087 leaves from 430 plant patches of *C. erecta* belonging to 16 sites of Córdoba city were analysed. The number of mines per leaf varied from 1 to 20 (mean  $\pm$  SE =  $2.56 \pm 0.04$ ), whereas host density ranged from 0.000037 to 0.84 mines  $\text{cm}^{-2}$  (mean  $\pm$  SE =  $0.038 \pm 0.076$ ) at the patch level and from 0.001 to 0.027 mines  $\text{cm}^{-2}$  (mean  $\pm$  SE =  $0.0085 \pm 0.0068$ ) at the site level. Of 5600 adults obtained (flies and parasitoids), 50.80% were parasitoids representing 20 species of Hymenoptera (further details in Fenoglio *et al.* 2010, 2013). The contribution of the most abundant species to overall parasitism was as follows: *C. flacilla* (11%), *Chrysocharis* sp. A (9.5%), *C. vonones* (8.27%) and *Chrysonotomyia* sp. B (8.33%).

Overall parasitism rates of *L. commelinae* at leaf scale ranged from 0 to 1 (mean  $\pm$  SE =  $0.53 \pm 0.01$ ), and responded to variations in the number of mines per leaf but not to host-density changes at patch or site level (Fig. 2). Parasitism rates decreased with increasing number of mines per leaf. Leaves with one mine had on average a parasitism rate around 50% compared to 30% on leaves with 10 mines.

Regarding the other variables included in the model, parasitism rates at leaf scale increased with increasing patch size and decreasing patch isolation; patch size was the strongest predictor variable according to our model (Table 1). We found no significant effects on parasitism at the site level (Table 1). The analysis of parasitism by parasitoid species separately showed consistent results with the response at the community level (see Table 1).

## DISCUSSION

Our hypothesis that changes in host density at different scales would influence the probability that *L. commelinae* would be parasitized at one particular



**Fig. 2.** Relationship between overall parasitism rates and (a) number of mines per leaf, (b) mine density per patch and (c) mine density per site. Grey lines represent the estimated models for each plant patch at the leaf level. Black line represents the estimated model mean.

**Table 1.** Estimated parameters obtained from the multi-level model testing the effect of several predictor variables on parasitism rates for: (a) the parasitoid community; (b) *Chrysocharis vonones*; (c) *Chrysocharis* sp A; (d) *Chrysocharis flacilla* and (e) *Chrysonotomiya* sp B

Parasitism by	Predictor variable	Mean	95% credible interval		Rhat	n.eff
(a) Community						
Leaf level	Host density	<b>-0.073</b>	<b>-0.142</b>	<b>-0.007</b>	<b>1.001</b>	<b>50 000</b>
	Host density	-0.099	-0.417	0.215	1.001	50 000
Patch level	Size	<b>0.446</b>	<b>0.152</b>	<b>0.738</b>	<b>1.001</b>	<b>50 000</b>
	Isolation	<b>-0.268</b>	<b>-0.448</b>	<b>-0.082</b>	<b>1.001</b>	<b>50 000</b>
Site level	Host density	-0.135	-0.803	0.524	1.001	28 000
	Urbanization index	0.136	-0.490	0.778	1.001	25 000
	Size	-0.007	-0.713	0.717	1.001	25 000
(b) <i>Chrysocharis flacilla</i>						
Leaf level	Host density	<b>-0.448</b>	<b>-0.577</b>	<b>-0.323</b>	<b>1.001</b>	<b>24 000</b>
	Host density	0.117	-0.316	0.555	1.001	36 000
Patch level	Size	<b>0.509</b>	<b>0.080</b>	<b>0.944</b>	<b>1.001</b>	<b>28 000</b>
	Isolation	0.059	-0.195	0.305	1.001	50 000
Site level	Host density	-0.342	-1.077	0.380	1.001	45 000
	Urbanization index	0.569	-0.128	1.283	1.001	50 000
	Size	-0.159	-0.972	0.644	1.001	50 000
(c) <i>Chrysocharis vonones</i>						
Leaf level	Host density	<b>-0.455</b>	<b>-0.595</b>	<b>-0.318</b>	<b>1.001</b>	<b>50 000</b>
	Host density	0.082	-0.325	0.487	1.001	50 000
Patch level	Size	<b>0.507</b>	<b>0.106</b>	<b>0.899</b>	<b>1.001</b>	<b>33 000</b>
	Isolation	-0.178	-0.396	0.035	1.001	50 000
Site level	Host density	-0.254	-0.827	0.327	1.001	18 000
	Urbanization index	0.165	-0.378	0.697	1.001	26 000
	Size	-0.313	-0.913	0.296	1.001	18 000
(d) <i>Chrysocharis</i> sp A.						
Leaf level	Host density	<b>-0.189</b>	<b>-0.318</b>	<b>-0.067</b>	<b>1.001</b>	<b>20 000</b>
	Host density	0.023	-0.333	0.383	1.001	50 000
Patch level	Size	<b>0.418</b>	<b>0.076</b>	<b>0.771</b>	<b>1.001</b>	<b>50 000</b>
	Isolation	<b>-0.208</b>	<b>-0.407</b>	<b>-0.010</b>	<b>1.001</b>	<b>20 000</b>
Site level	Host density	0.002	-0.755	0.731	1.001	50 000
	Urbanization index	-0.251	-0.953	0.434	1.001	50 000
	Size	0.247	-0.528	1.035	1.001	50 000
(e) <i>Chrysonotomiya</i> sp B						
Leaf level	Host density	<b>-0.273</b>	<b>-0.411</b>	<b>-0.136</b>	<b>1.001</b>	<b>47 000</b>
	Host density	0.415	-0.111	0.926	1.001	50 000
Patch level	Size	0.352	-0.108	0.827	1.001	50 000
	Isolation	-0.228	-0.510	0.058	1.001	26 000
Site level	Host density	0.130	-0.779	1.151	1.001	50 000
	Urbanization index	0.375	-0.560	1.385	1.001	34 000
	Size	-0.294	-1.372	0.753	1.001	43 000

For each parameter, we present the mean value and 95% credible intervals (95% CI). Rhat is the potential scale reduction factor and indicates the MCMC convergence (at convergence Rhat = 1). n.eff is a measure of effective sample size. All values are presented in logit scale. Variables with 95% CI not overlapping zero are shown in bold.

scale was not fully supported, since we found that only the number of mines at the leaf scale was important. Both overall parasitism and parasitism by dominant parasitoid species were inversely related to host density at this scale. Two variables associated with host habitat – plant patch size and isolation – had a stronger effect than leaf host density in determining overall parasitism rates. These results suggest that the realized parasitism caused by the community of parasitoids here studied is sensitive to hierarchical cues, but not necessarily related to host density.

Variation from leaf to leaf in the number of mines was the only host-density variable that proved to be important for parasitism in *L. commelinae*. Larvae in leaves with many leaf mines were less likely to be parasitized than larvae in leaves with few conspecifics, which was contrary to our expectation of observing direct density dependence. However, this result is consistent with a recent meta-analysis that has shown that the correlation between parasitism and host density was more often negative where the host belonged to orders Lepidoptera or Diptera, or where the study was conducted at a finer grain size (Gunton & Pöyry

2016). Interestingly, this inverse pattern was found at both levels of parasitism of *L. commelinae*: by the whole parasitoid community and by the dominant species. Although parasitism by multiple parasitoid species could lead to a confounding effect in the detected outcome of density dependence (Brown 1989), here we found a clear pattern for the community, which seems to be the result of the action of the dominant species. The similarity in the response to host density found across parasitoid species disagrees with previous works that have demonstrated a differential response of species to host density mainly due to differences in habitat use and morphological and life history characteristics (Doak 2000; Norowi *et al.* 2000; Costamagna *et al.* 2004; Woodcock & Vanbergen 2008). Nevertheless, our result provides an opportunity to further explore the patterns of species occurrence, considering that they exploit this host similarly in terms of density and that they have similar host ranges (Salvo & Valladares 1997), which makes them potential competitors.

Different mechanisms have been proposed to explain an inverse relationship for single parasitoid species, such as limitations imposed by handling time or egg availability, interference among individuals, and occurrence of a 'spreading the risk' behaviour (Walde & Murdoch 1988; Visser *et al.* 1999; Hassell 2000). In addition, other mechanisms have been invoked to explain the observed pattern in concealed hosts, such as less efficiency of parasitoids when searching for leaves with multiple hosts due to coalescence of mines (Connor *et al.* 1999) or avoiding leaves previously visited by other parasitoids due to presence of chemical marks (Hoffmeister & Roitberg 1997).

Regarding the number of mines per patch, we found that was irrelevant to the probability that the herbivore would be attacked on a leaf. It seems that female parasitoids of *L. commelinae* are capable of distinguishing and selecting at distance large patches of *C. erecta*, independently of the amount of resources on them. Patch size may represent a strong stimulus for the parasitoid species here studied since it had a powerful positive effect on the parasitism at leaf scale by three of the four species analysed. Host density at the large spatial scale did not influence parasitism in *L. commelinae*. If there were a higher number of mined patches in the neighbourhood, this neither increased the probability of parasitoids discovering them nor enhanced the rate of attacks through population level response (Ives *et al.* 1993; Philpott *et al.* 2009). However, the density of *L. commelinae* at the site level did not affect the probability of the leaf miners becoming parasitized. The urbanization index was also irrelevant for parasitism rates per leaf, as previously shown for site level parasitism (Fenoglio *et al.*

2009), discarding a potential negative effect of urban disturbance on parasitoid attack.

Our approach to analysing the density–parasitism relationship is a novel one since it incorporates scale explicitly and it considers how parasitoids forage in a patchy environment (Pierre & Green 2008). Hierarchical linear models are a powerful tool to test and draw inference from measured associations between variables across scales in ecological systems (McMahon & Diez 2007). Here, we proved that it could be useful to evaluate the response of parasitoids to host-density variation at multiple spatial scales. We are aware that our work has limitations to identify mechanisms by which spatial patterns of parasitism are generated, mainly because parasitoid foraging behaviour is not always correlated with parasitism patterns (Rosenheim *et al.* 1989; Heimpel & Casas 2008). In addition, our data consider realized parasitism rates, underestimating the actual mortality caused by parasitoids. However, the hierarchical Bayesian approach allowed us to detect that variables at different scales, regarding host and host habitat, had an effect on parasitism per leaf in *L. commelinae*.

In summary, a higher number of *L. commelinae* larvae were attacked when host density per leaf was low. This inverse spatial density dependence seems to be a common phenomenon in the analysed parasitoid community. Our study demonstrated that dominant species from a diverse assemblage of parasitoids contributed to variation in overall parasitism mainly through a spatial density response. Polyphagous parasitoids are expected to respond better to changes in the general density of several host species than specialist parasitoids (Stiling 1987). However, even when the parasitoid complex here studied consists of polyphagous species (Valladares & Salvo 1999), female parasitoids probably focus on cues provided by *L. commelinae*, one of the most abundant resources in the urban habitat (Fenoglio *et al.* 2013). Inverse density dependence may have a stabilizing influence on host–parasitoid interactions (Hassell 1985) when a partial refuge that prevents parasitoids from driving the host population to extinction is created (Cronin 1989). In fact, the leaves with high *L. commelinae* density may represent a partial refuge offering an enemy-free space for the leaf miner that may be in part compensating the negative impact of intra-specific competition in leaves with multiple mines (Aparicio *et al.* 2015). However, a temporal study is needed to determine the relevance of inverse density dependence in parasitism in promoting stability in *L. commelinae* parasitoid interactions.

All in all, our research highlights the idea that hierarchical multi-scale analyses are useful to fully understand how parasitism is linked to host density and host habitat variables in highly heterogeneous systems. Moreover, our results illustrate the importance

of considering the spatial dimension when assessing host–parasitoid interactions in real landscapes. Nonetheless, experiments on movement behaviour, manipulation of landscape structure, and spatially realistic models would be necessary to advance in this area of study (Cronin & Reeve 2014).

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Appendix S1.** Bugs code for the fitted model.