

# Looks are important: parasitic assemblages of agromyzid leafminers (Diptera) in relation to mine shape and contrast

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## Summary

1. We test the hypothesis that leaf mine appearance can affect the risk of leafminers being discovered by parasitoids, and therefore influence parasitic assemblages, using a comparative study of parasitic complexes associated with 28 agromyzid species in Central Argentina. Analyses were based on size, structure (defined as the number of species in host-range categories) and impact (percentage parasitism) of parasitic complexes on leafminers. Mine appearance was defined in terms of shape (linear, linear-blotch, blotch) and colour (high or low contrast with the leaf lamina).
2. Irrespective of the agromyzid species involved, significant differences were found in the structure of the parasitoid complexes: specialists were more abundant and generalists rarer than expected in blotch and cryptically coloured mines.
3. There were no differences in average parasitoid species richness and parasitism rates among differently coloured or shaped galleries. However, mine appearance significantly affected parasitic assemblage structure, with shape driving generalist species richness and contrast influencing that of specialists. Mine shape also affected parasitism rates, which were highest for generalists in linear mines, and for specialists in blotch mines. The existence of a gradient of discovery from the cryptically coloured blotch mines to the most apparent highly contrasting linear ones was supported by significant correlations of this gradient with richness and parasitism rates of generalist and specialist parasitoids.
4. Taxonomic composition of parasitic complexes (analysed through parasitoid species abundance) was separated significantly according to host mine shape. An even more significant classification of assemblages was achieved when the combination of mine shape and colour was considered in the discoverability gradient.
5. Our results suggest that despite leaf mines being an ecologically homogeneous resource, their morphology might offer varying degrees of refuge against different parasitoids.

*Key-words:* community structure, host–parasitoid interactions, leaf mine morphology, parasitoid host range, primary defences.

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## Introduction

Herbivore insects vary enormously in their susceptibility to parasitoids, and a number of studies have focused on the ecological determinants of such variation, both in terms of species richness of parasitoid communities and of their impact on the host populations (e.g. Askew

1980; Sato 1990; Mills 1993; Hawkins 1994; Dawah, Hawkins & Claridge 1995; Porter & Hawkins 1998). Variation in insect vulnerability to parasitoids seems related mainly to host characteristics that provide protection against the search, attack or development of parasitoids (Gross 1993). Both number of parasitoid species as well as the structure of parasitic complexes can be affected by host phylogenetical and ecological properties, revealing evolutionary aspects of host–parasitoid interactions (Pschorn-Walker & Altenhofer 1989; Hoffmeister 1992).

Feeding niche has been identified as the major determinant of parasitoid community size and structure

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(Hawkins 1994). Leaf mining larvae consume live foliage while simultaneously dwelling inside it (Connor & Taverner 1997). Damage symptoms are visible as pale marks (mines) on the leaf surface, where the parenchyma tissue has been consumed, leaving an internal tunnel covered by the intact epidermic layers. Leafminers support more species of parasitoids per host species than insects in any other feeding niche, and tend to suffer the highest rates of parasitism (Hawkins, Cornell & Hochberg 1997). The high susceptibility of leafminers to parasitoids has been attributed to the lack of mobility of the larvae, coupled with the high visibility of the mines and the scant physical protection offered by the leaf epidermis (Hawkins 1994). Such vulnerability could also arise from a great ecological homogeneity among leafminers resulting from the strong selection pressures exerted by feeding within a leaf lamina (Godfray 1994).

Within each feeding niche, parasitoid diversity can vary as a function of specific factors, which seem related generally to host apparency (e.g. Hawkins & Goeden 1984; Hawkins & Gagne 1989; Hoffmeister 1992; Dawah *et al.* 1995; Porter & Hawkins 1998). In the particular case of organisms that leave feeding tracks, such as leafminers, the pattern of such tracks can affect the risk of parasitoid attack (Kato 1985) and therefore could have profound effects on the associated parasitic communities. Leaf mines can take different shapes, this trait being characteristic of each species (Spencer & Steyskal 1986). When mining larvae advance in only one direction a linear mine is produced; a blotch mine results when the larva eats in several directions; an intermediate type, the linear-blotch mine, is observed when a larva starts forming a short linear mine which later expands into a blotch (Hering 1951).

The mine itself could actually represent the most important clue for parasitoids in locating host miners (Vinson 1976). Field observations and laboratory experiments suggest that mines are detected by parasitoids in flight, apparently following visual stimuli (Casas 1988; Sugimoto, Kawado & Tadera 1988a). After alighting, parasitoids have been proved to follow vibratory and chemical, as well as visual stimuli (Casas *et al.* 1998; Djemai, Meyhöfer & Casas 2000). Mine shape could influence not only the risk of a mine being found by a parasitoid, but also the risk of the host itself being located after its mine has been discovered: larvae in linear mines are found generally at the widest end of the tunnels, and parasitoids looking for hosts have been observed to backtrack if the linear mine begins to narrow (Sugimoto *et al.* 1987; 1988a,b).

Some mine shapes are reported to act as an anti-parasitoid strategy by confusing parasitoids (Kato 1984). In contrast to the unmistakable linear galleries, blotch mines may be confused with dead areas of leaves or other damage symptoms (e.g. plant pathogens). Moreover, a blotch mine offers a wider space for movement, allowing larval and pupal defensive behaviour (Bacher *et al.* 1997) which may increase parasitoid searching

time considerably (Djemai *et al.* 2000). Consequently, hosts in blotch mines could be expected to suffer lower parasitization than those in linear mines. The degree of visual contrast provided by the colour of the mine, which can vary from a striking white to a more cryptic light green or brown, could also act as a camouflage against parasitoids. Despite these interesting possibilities, relatively little attention has been paid to the potential influence of mine appearance on susceptibility to parasitism (Hespenheide 1991). Most studies on this subject have dealt with population and behavioural aspects, without considering the consequences that mine morphology may have on the associated parasitic communities.

We ask whether there is a relationship between mine aspect and traits (species richness, structure, parasitism rates) of parasitoid assemblages on Agromyzidae (Diptera) leafminers in Central Argentina. Agromyzid flies are one of the largest taxonomic groups containing leaf mining larvae, and well represented in the study area where they support a rich parasitoid community (Salvo & Valladares 1998, 1999). Our hypothesis is that mine apparency, as characterized by shape and contrast against the leaf lamina, represents a primary defence affecting the probability of leafminers being discovered, and therefore influencing parasitoid assemblage composition.

## Materials and methods

### STUDY SITE, SAMPLING AND REARING TECHNIQUES

Samples were collected from 1991 to 1995 at 30 localities – including natural, urban and agricultural habitats – in Córdoba province, Central Argentina. All mined leaves, independent of appearance, were collected from every available plant along transects in each locality. The leaves were cut and placed in plastic bags, transported to the laboratory, and kept until flies and parasitoids emerged (Salvo & Valladares 1998). Special rearing techniques were developed for leaves with high water content or those that rapidly dried once excised. In the former case, either a box covered with gauze was used instead of a bag, or the mined portion of the leaf was cut off from the rest, to reduce the risk of the mine becoming infected with mould. In the second case either stems with several leaves were collected, thus slowing desiccation on individual leaves, or humid cotton-wool pieces were added to the bags. Adults of both leafminers and parasitoids were stored in glass vials plugged with cotton-wool; once emergence had ceased they were counted and identified. Techniques to assess rearing success are labour-intensive, so it was not logistical to apply them to the large number of hosts and parasitoid considered in this study.

Reference collections of both Agromyzidae and parasitoids were deposited in Cátedra de Entomología, Universidad Nacional de Córdoba, Argentina.

DATA SET AND PARASITOID SPECIES  
CATEGORIZATION

Parasitic complexes of 28 species of Agromyzidae with sample sizes of over 100 reared adults were analysed according to their mine attributes (Appendix I). A total of 60 parasitic species belonging to five taxonomic families were associated with these leafminers, and 39 of them (those with more than 10 reared adults) were considered in the present study (Appendix II).

The structure of parasitic complexes is often examined focusing on the number of generalist and specialist species contained within them. A regional survey revealed very broad host ranges for parasitic species, each attacking an average of 10 from a total number of 51 agromyzid host species (Salvo & Valladares 1999). Given this feature of the system, parasitic species were assigned to one of five host-range categories (Appendix I): (1) parasitoids associated with a unique host (henceforth referred to as 'specialists'); (2) parasitoids attacking two to four hosts; (3) species associated with five to eight hosts; (4) those with nine to 16 hosts; and (5) parasitoids associated to 17 or more hosts (henceforth called 'generalists'). This categorization applies to the parasitoid community associated with agromyzid hosts. Preliminary surveys of parasitoids on non-agromyzid leafminers in the study area suggest that our 'specialists' do not feed on other taxonomic groups of leafminers, although at present this possibility cannot be ruled out completely (Salvo & Valladares 1999).

The association between taxonomic family and host-range categories was examined using an exact *G*-test.

HYPOTHESIS, PREDICTIONS AND  
STATISTICAL ANALYSIS

We can hypothesize that it would be more difficult for parasitoids to detect either the mine itself or the larva within when hosts develop in blotch mines (B); this mine shape could represent an antiparasitoid strategy. Thus, leafminers constructing blotch mines would be expected to support fewer parasitoid species and suffer lower parasitism rates than those in linear galleries (L). This would result from a reduction in the generalist parasitoid component, which would be less efficient in recognizing and exploiting this mine type, whereas specialists would be adapted to find their hosts, independently of mine shape. Hosts in linear-blotch mines (LB) should show intermediate values. Mine colour could have a similar effect, with the cryptically coloured (CC) brown or greenish mines suffering lower parasitoid attack – mainly by supporting fewer generalists – than white or yellow mines which offer a higher contrast (HC) against the leaf lamina.

Given the comparative nature of this study, a possible dependence of mine attributes on host phylogeny (Godfray 1994) cannot be ruled out completely. The distribution of shape and contrast of the mines among

agromyzid genera was tested for independence through the exact *G*-test.

To obtain a general picture of community structure, avoiding the bias of any particular host–parasitoid associations, data of parasitoids emerging from each mine type were pooled. The possible dependence of the number of parasitic species and individuals (in each category) on mine shape and contrast was assessed using a  $\chi^2$  test for individuals and an exact *G*-test for species. The adjusted residuals were also analysed (Agresti 1996).

The effect of mine shape and contrast on the 28 parasitic complexes associated with the agromyzid species was analysed by two-way ANOVA, and a posteriori the Tukey test. The following variables were considered: size (total species richness); structure, defined by the number of species in each category and by the generalist species ratio (number of generalist species divided by total number of parasitic species); and percentage parasitism, total (number of parasitoids divided by total number of adults reared) and for each category (number of parasitoids in a given category divided by total number of parasitoids reared). Data from parasitoid species richness were log-transformed; percentages of parasitism and generalist species ratios were transformed angularly in order to meet the assumptions of normality and homogeneity of variance. All data are presented as backtransformed means  $\pm$  SD.

Because sampling effort was uniform for all leafminer species, a greater sample size for a given species reflects greater abundance in the field. Biological factors and artefactual effects due to sample size are therefore probably linked. Any statistical procedure to eliminate the effect of sample size on species richness could simultaneously eliminate real biological differences and might lead to erroneous conclusions. We therefore consider the actual number of parasitic species reared as the best way to measure species richness. Nevertheless, possible sample size effects were incorporated in the analysis by performing an ANCOVA on the above-mentioned variables and including the total number of adults collected (log) as covariate. This method has been mentioned recently as being more appropriate than the use of regression residuals (Freckleton 2002).

Results from the two-way ANOVA were used to devise a proxy variable representing a gradient in discoverability. Variations in number of parasitoid species and percentage parasitism (total and for each parasitic category) on agromyzid hosts were examined in relation to the gradient of discoverability using the non-parametric Spearman's rank correlation test.

The influence of mine shape, contrast and their combination on parasitoid community composition was also examined through multivariate analysis of variance (MANOVA) and discriminant analysis (DA) based on the Wilks's lambda statistic on the standardized abundances of generalist and specialist parasitoid species on each host.

## Results

### GLOBAL ANALYSIS OF THE PARASITOID COMMUNITY

About half the 28 leafmining species, and 64% of the total 40 347 adults examined for this study, were reared from linear mines; blotch mines (six species, and 4% of the specimens, respectively) were the least abundant resource, whereas linear-blotch mines occupied an intermediate position, with nine species and 32% of the individuals (Appendix II). White mines were more abundant (17 species, 64% specimens) than those showing a less contrasting colouration (11 species, 36% specimens). Independence of mine attributes from agromyzid generic affiliation was shown for mine colour ( $G = 13.96$ , d.f. = 8,  $P > 0.05$ ), but not for mine shape ( $G = 33.71$ , d.f. = 16,  $P = 0.002$ ). The strongest deviations from values expected by a random distribution were represented by an excess of blotch mines in the genus *Calycomyza*, and of linear mines in *Liriomyza* (Appendix II).

Only six of the 39 parasitic species were reared exclusively from one leafminer species, whereas 10 fed on 17 or more hosts, with the remaining species showing intermediate host ranges (Appendix I). Parasitoid categories based on host ranges were not associated with taxonomic affiliation ( $G = 11.86$ , d.f. = 12,  $P > 0.05$ ).

When data were pooled according to mine attributes (irrespective of host identity) the number of parasitoid species – in each host range interval – associated with the different mine types appeared to be independent of either shape or contrast ( $G = 2.43$ , d.f. = 8 for shape;  $G = 2.67$ , d.f. = 4 for contrast;  $P > 0.05$ ); however, a noticeable concentration of specialists was observed on cryptically coloured mines (Table 1). Abundance of parasitoids from each host-range interval was not independent of mine morphology ( $\chi^2 = 3208.84$ , d.f. = 8,

$P < 0.0001$ ) or contrast ( $\chi^2 = 365.38$ , d.f. = 4,  $P < 0.0001$ ). Particularly strong departures from expected values were observed for specialists and generalists: the former were significantly more abundant, and generalists more rare than expected, in blotch and cryptically coloured mines (Table 1). Total parasitoid abundance followed that of their resources, being greatest in linear and highly contrasting mines and lowest in blotch and cryptically coloured ones.

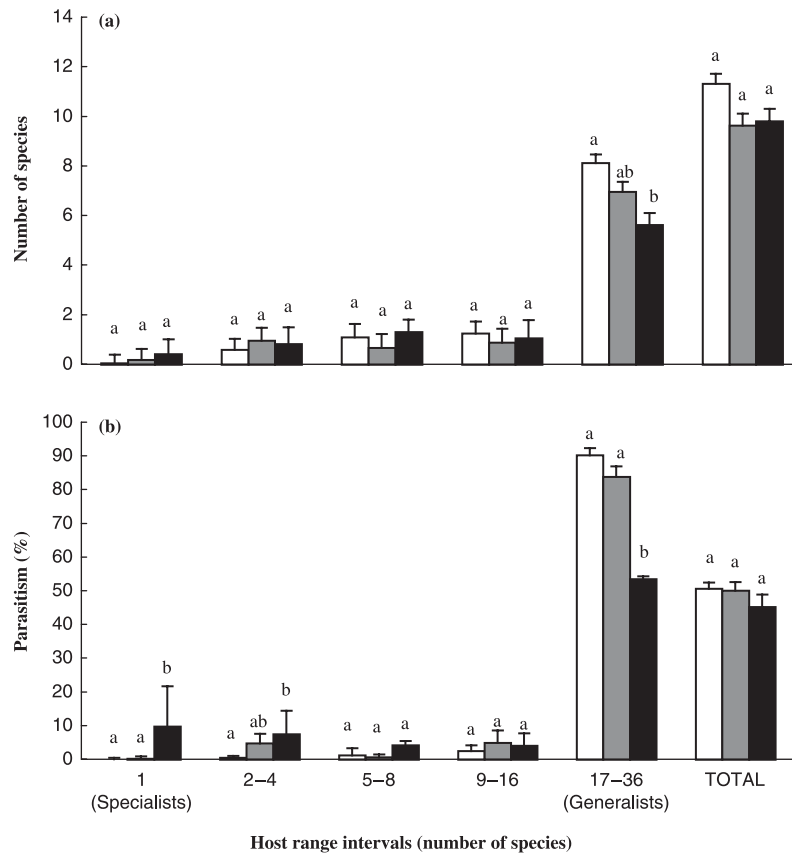
### SIZE AND STRUCTURE OF PARASITIC COMPLEXES

Neither average species richness of parasitic assemblages associated with leafminer species nor the richness of parasitoids in most host-range intervals (Figs 1a, 2a) varied significantly with either mine or contrast (ANOVA,  $P > 0.05$ ). However, leafminers in blotch galleries supported significantly fewer markedly generalist parasitoid species (ANOVA,  $F_{2,22} = 5.99$ ,  $P = 0.008$ ) than hosts in linear mines (Fig. 1a), whereas more specialist parasitoids were found on hosts developing in cryptically coloured mines (Fig. 2a) (ANOVA,  $F_{1,22} = 7.19$ ,  $P = 0.01$ ). The generalist species ratio was very similar on hosts constructing linear ( $X = 0.75$ , SE = 0.01,  $n = 13$ ) or linear-blotch ( $X = 0.72$ , SE = 0.008,  $n = 9$ ) mines, but lower on blotch ( $X = 0.57$ , SE = 0.002,  $n = 6$ ) mines, although such differences were not statistically significant (ANOVA,  $P > 0.05$ ). There were no significant differences between generalist species ratio from highly contrasting or cryptically coloured mines (ANOVA,  $P > 0.05$ ) and no significant shape  $\times$  contrast interaction was found for either species richness or parasitism rate for any parasitoid host range intervals (ANOVA,  $P > 0.05$  in all cases).

When ANCOVA analyses were performed to assess the effects of sample size on parasitoid species richness, the preliminary test for homogeneity of slopes revealed no

**Table 1.** Total number of species and number of individuals obtained from each mine type. The expected figures from a random distribution are indicated in brackets. \*Values significantly deviated from those expected according to adjusted residuals ( $\chi^2$  test  $P < 0.0001$ )

Parasitoid host range	Shape			Contrast	
	Linear	Linear-blotch	Blotch	High	Low
Species richness					
Specialists ( $n = 6$ )	1 (2)	2 (2)	3 (2)	1 (3)	5 (3)
2–4 hosts ( $n = 12$ )	9 (7)	6 (6)	5 (6)	10 (9)	10 (11)
5–8 hosts ( $n = 7$ )	7 (6)	5 (5)	5 (5)	7 (7)	7 (7)
9–16 hosts ( $n = 4$ )	4 (4)	3 (3)	4 (3)	4 (4)	4 (4)
Generalists ( $n = 10$ )	10 (11)	10 (9)	9 (9)	10 (9)	10 (11)
Total ( $n = 39$ )	31	26	26	32	36
No. of individuals					
Specialists	11* (116)	35 (46)	122* (6)	20* (112)	148* (56)
2–4 hosts	93* (300)	293* (118)	49* (16)	197* (289)	238* (146)
5–8 hosts	131* (171)	55 (68)	62* (9)	123* (165)	125* (83)
9–16 hosts	298* (403)	244* (159)	42* (22)	370 (388)	214 (196)
Generalists	10 547* (10 089)	3741* (3977)	328* (549)	9 964* (9720)	4652* (4896)
Total	11 229	4403	613	10 674	5377



**Fig. 1.** Mean ( $\pm$  SE) parasitoid species richness (a) and percentage parasitism (b) for total parasitoids and for each host range interval, on agromyzids with differently shaped mines: linear (open bars), linear-blotch (grey bars) and blotch (black bars). Different letters within each group of bars indicate significant differences among means (Tukey test  $P < 0.05$ ).

interaction between sample size and mine shape or contrast (Table 2). In the final model (with the interaction term removed), sample size affected all categories except specialists. Thus we can conclude that, even accounting for sample size, generalist richness was affected by mine shape ( $F_{2,25} = 4.48$ ,  $P = 0.02$ ), as described above (adjusted means after ANCOVA: linear  $X = 7.93$ , linear-blotch  $X = 6.87$ , blotch mines  $X = 5.76$ ).

Total percentage parasitism (Figs 1b and 2b) showed no significant variations among hosts with different-shaped or coloured mines ( $P > 0.05$  in both cases). However, relative parasitism rates from generalist parasitoids were significantly higher ( $F_{2,25} = 17.97$ ,  $P < 0.0001$ ) when hosts developed in linear mines, whereas the representation of parasitoids with narrower host ranges increased (specialists:  $F_{2,25} = 6.18$ ,  $P = 0.007$ ; host range 2-4:  $F_{2,25} = 4.35$ ,  $P = 0.03$ ) in blotch mines (Fig. 1b). Mine colour (Fig. 2b) did not affect parasitism rates in any host-range interval ( $P > 0.05$  in all cases).

#### DISCOVERABILITY GRADIENT

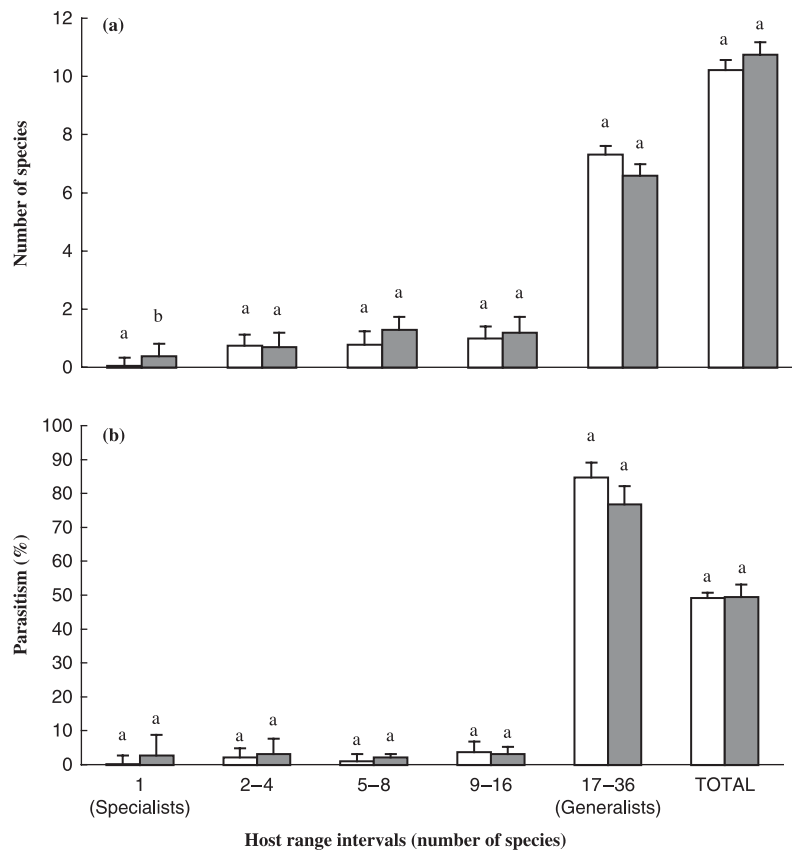
With mine shape impacting most on the structure of parasitic complexes, we proposed a gradient of increasing mine discoverability in which shape was the driving factor, with contrast as the second force. Mines were classified and ordered as follows: 1-BCC, 2-BHC,

3-LBCC, 4-LBHC, 5-LCC, 6-LHC (e.g. BCC represents blotch mine, cryptically coloured).

Data from the extreme parasitoid host-range categories were correlated significantly ( $P < 0.01$ ) to this gradient (Fig. 3). Specialist diversity ( $r = -0.47$ ) and abundance ( $r = -0.49$ ) increased as mines and hosts become more difficult to detect. Generalists showed an inverse relationship, becoming more diverse ( $r = 0.48$ ) and causing higher parasitism rates ( $r = 0.64$ ) at increasing host discoverability. The generalist species ratio was also correlated positively with the discoverability gradient ( $r = -0.38$ ,  $P = 0.04$ ). Total diversity or abundance of parasitoids, as well as those of species in intermediate host-range categories, were not related to the above gradient ( $P > 0.05$  in all cases).

#### PARASITOID COMMUNITY COMPOSITION AT THE SPECIES LEVEL

A multiple analysis of variance on the specific composition (relative abundance of each parasitoid species) of parasitic assemblages associated with agromyzid hosts revealed a significant effect of mine shape (Wilks's  $\lambda < 0.001$ ,  $F_{2,50} = 24.91$ ,  $P = 0.04$ ) but not of contrast (Wilks's  $\lambda = 0.147$ ,  $F_{1,26} = 0.22$ ,  $P = 0.96$ ). The combination of shape and contrast provided a more significant differential than shape by itself (Wilks's  $\lambda < 0.001$ ,  $F_{10,110} = 5.62$ ,  $P = 0.003$ ).



**Fig. 2.** Mean ( $\pm$  SE) parasitoid species richness (a) and parasitism (b) for total parasitoids and for each host range interval on agromyzids from mines with different degree of contrast: highly contrasting (open bars) and cryptically coloured mines (grey bars). Different letters indicate significant differences between means in each pair of bars (Tukey test  $P < 0.05$ ).

The degree of divergence among assemblages was shown by discriminant analyses (DA). When each factor was considered separately, only mine shape provided a significant group discrimination (function 1: eigenvalue = 38.99, variance explained = 69%, canonical correlation = 0.987, Wilks's  $\lambda = 0.001$ ,  $\chi^2 = 95.59$ , d.f. = 46,  $P < 0.0001$ ; function 2: eigenvalue = 17.64, variance explained = 31%, canonical correlation = 0.973, Wilks's  $\lambda = 0.054$ ,  $\chi^2 = 40.96$ , d.f. = 22,  $P = 0.008$ ). The first function, with the highest explanatory power, ordered the parasitic assemblages as expected, by placing the linear-blotch mines – which share characteristics from the other two types – in an intermediate position (Fig. 4). The model provided 100% correct classification.

Although mine contrast by itself did not reveal significant effects on parasitoid assemblages, DA based on its combination with mine shape (Fig. 5) clearly differentiated the six groups. Three significant functions absorbed 96% of variance (function 1: eigenvalue = 141.36, variance explained = 74%, canonical correlation = 0.996, Wilks's  $\lambda < 0.0001$ ,  $\chi^2 = 188.01$ , d.f. = 105,  $P < 0.0001$ ; function 2: eigenvalue = 26.40, variance explained = 14%, canonical correlation = 0.982, Wilks's  $\lambda = 0.0001$ ,  $\chi^2 = 121.07$ , d.f. = 80,  $P = 0.002$ , function 3: eigenvalue = 14.30, variance explained = 8%, canonical correlation = 0.967, Wilks's  $\lambda = 0.003$ ,  $\chi^2 = 76.37$ , d.f. = 57,  $P = 0.04$ ). Again assemblages were classified into their correct group with 100% accuracy. Parasitic assemblages

associated with hosts in highly contrasting blotch and linear-blotch mines were dramatically different from the remaining assemblages by the first function, whereas the second function separated cryptically coloured blotch and linear-blotch mines. Hosts in linear mines supported the most homogeneous assemblages irrespective of mine contrast.

## Discussion

Observations from studies on parasitoid behaviour and specific leafminer–parasitoid interactions have shown that mine appearance can affect not only parasitoid success (e.g. Sugimoto 1977; Meyhofer, Casas & Dorn 1997; Djemai *et al.* 2000) but also act as an antiparasitoid strategy (Kato 1984). If such effects were translated into higher levels of organization, such as the community, mining patterns could be expected to influence the parasitoid assemblages associated to leafminers.

Contrary to our expectations, we did not find differences in the total number of parasitic species associated with mines varying in form or colour, neither from a global community analysis (pooling data from all host species sharing the same mine type) nor from a more detailed comparison of parasitic assemblages on each host. Parasitism rates on the studied agromyzid species were also independent of mine attributes. The lack of

**Table 2.** Summary of statistics obtained from analysis of covariance, performed with species richness (log) of parasitoid categories as dependent variables, shape or contrast of mines as main factor and sample size (log) as covariate

Species richness	Interaction <sup>1</sup> /main factor <sup>2</sup> /covariate <sup>3</sup>	F	d.f.	P
Total	Shape × sample size	1.497	2, 22	0.246
	Shape	0.690	2, 24	0.510
	Sample size	13.997	1, 24	0.001
	Contrast × sample size	2.091	1, 24	0.161
	Contrast	0.001	1, 25	0.976
	Sample size	14.365	1, 25	0.001
Specialists	Shape × sample size	0.698	2, 22	0.508
	Shape	1.734	2, 24	0.187
	Sample size	0.051	1, 24	0.823
	Contrast × sample size	0.011	1, 24	0.917
	Contrast	7.439	1, 25	0.012
	Sample size	0.328	1, 25	0.572
2–4 hosts	Shape × sample size	1.187	2, 22	0.324
	Shape	0.924	2, 24	0.444
	Sample size	6.237	1, 24	0.020
	Contrast × sample size	0.224	1, 24	0.640
	Contrast	0.103	1, 25	0.751
	Sample size	5.321	1, 25	0.030
5–8 hosts	Shape × sample size	0.858	2, 22	0.438
	Shape	2.843	2, 24	0.059
	Sample size	16.015	1, 24	0.001
	Contrast × sample size	1.051	1, 24	0.316
	Contrast	1.110	1, 25	0.302
	Sample size	10.745	1, 25	0.003
9–16 hosts	Shape × sample size	2.239	2, 22	0.130
	Shape	0.433	2, 24	0.731
	Sample size	5.462	1, 24	0.028
	Contrast × sample size	2.521	1, 24	0.125
	Contrast	0.106	1, 25	0.747
	Sample size	4.976	1, 25	0.035
Generalists	Shape × sample size	0.633	2, 22	0.564
	Shape	4.473	2, 24	0.022
	Sample size	3.245	1, 24	0.084
	Contrast × sample size	0.267	1, 24	0.610
	Contrast	1.670	1, 25	0.208
	Sample size	5.931	1, 25	0.022

<sup>1</sup>All interactions proceed from preliminary models (main factor + covariate + interaction) for testing homogeneity of slopes. <sup>2,3</sup> Statistics for main factor (shape and contrast) and covariate (sample size) from the final model, with the interaction term removed.

influence of mine morphology on these general features of the parasitic communities agrees with the scarce evidence available on this issue (Askew & Shaw 1974; Askew 1994; Memmot, Godfray & Gauld 1994).

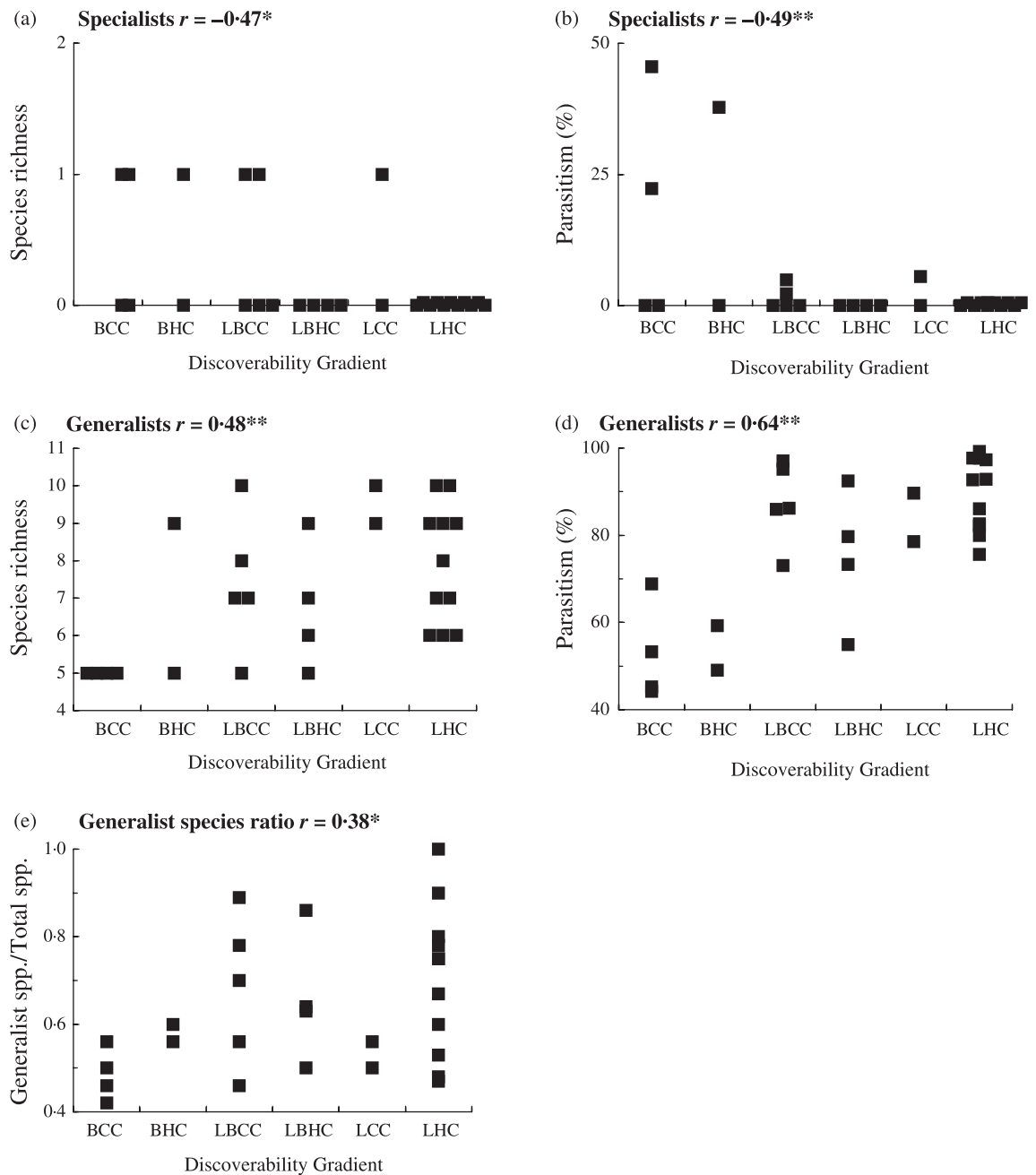
However, not all parasitoid species are equivalent (Hawkins & Mills 1996). Much relevant information is missing or even lost when parasitic assemblages are defined only in terms of number of species involved or total parasitism rates. The functional structure of a parasitoid community depends mainly on the host ranges of its members, and this information is essential to understanding the mechanisms driving parasitoid community organization (Hawkins 1994). Most parasitic species in the present system were generalists, characteristic of endophytic insects (Hawkins 1994). Particular specializations as well as generalism may be taxonomically conserved (Godfray 1994), and it must be noted that host ranges were not independent from parasitoid taxonomy (Salvo & Valladares 1999).

Ranking parasitoids according to their host ranges, from extreme specialists confined to one host species to

generalists recorded on 17–36 hosts, allowed analysis of the structure of the parasitic assemblages. Differences attributable to mine type were revealed, supporting the assertion that insect defences may not be equally effective against the entire range of natural enemies (Dyer & Gentry 1999).

Even a rough comparison of pooled data, independent of the host species involved, showed specialist parasitoid species concentrating on blotch and cryptically coloured mines – despite these being the least abundant resources – and generalists following the opposite pattern. The structure of parasitic assemblages seemed to be affected differentially by the two components of mine appearance: specialist species richness responded mainly to mine contrast, whereas generalists were driven by mine shape.

Besides being poorer in species number, blotch mines also suffered least from generalist parasitoids. Instead they showed a significantly greater proportion of parasitism by specialists. Mine shape could therefore be influencing both the possibility of colonization by



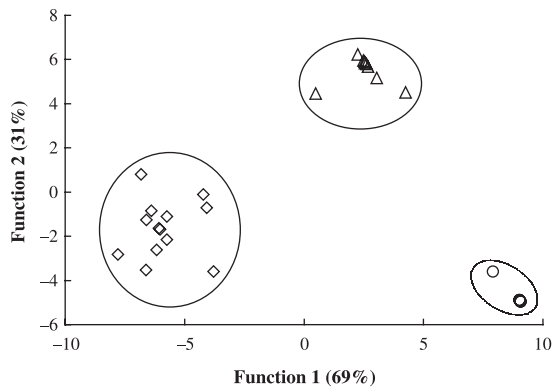
**Fig. 3.** Relationships between species richness or parasitism rates observed in the parasitic complexes and a discoverability gradient based on mine appearance. LHC = linear highly contrasting mines, LCC = linear cryptically coloured mines, LBHC = linear-blotch highly contrasting mines, LBCC = linear-blotch cryptically coloured mines, BHC = blotch highly contrasting mines, BCC = blotch cryptically coloured mines.  $r$  = Spearman's rank correlation coefficient,  $*P < 0.05$ ,  $**P < 0.01$ .

generalist or specialist parasitic species in evolutionary time, and their efficiency to parasitize their hosts in ecological time. The changes observed also indicate that pooling of different mortality factors may lead to inadequate conclusions (Zvereva & Kozlov 2000).

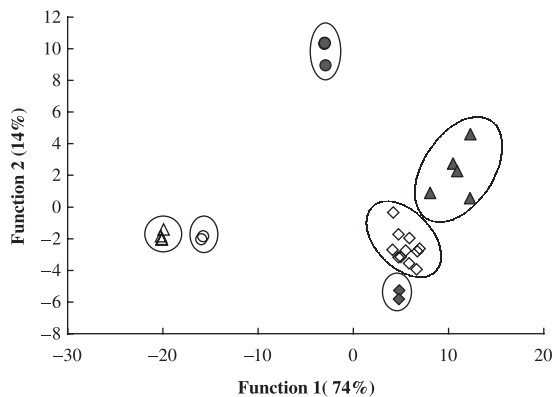
Blotch mines may be more difficult for generalist parasitoids to exploit because they offer reduced visual cues; also, generalists could be more diverse where host diversity is higher – i.e. linear mines – as there would be more room for resource partitioning (Askew & Shaw 1986). Whatever the mechanism leading to the prevalence of generalists in linear mines, this would

make the latter type especially unfavourable for specialists, which are frequently poor competitors (Zwölfer 1971; Askew & Shaw 1986). Therefore, the rarity of specialist parasitoids in linear mines, as well as their increase in blotch mines, could be attributed to the degree of competition from generalists. Competition does appear to be an important organizing force in parasitoid communities associated with endophytic hosts (Hawkins 1990), and it seems particularly plausible in the system studied given the extremely high overlap of parasitoid host ranges (Valladares, Salvo & Godfray 2001).





**Fig. 4.** Discrimination of parasitic assemblages based on mine shape (linear mines represented by diamonds, linear-blotch by triangles and blotch by circles), using the abundance of specialized (one to four hosts) and generalist (17 or more hosts) parasitoids for 28 agromyzid hosts in Central Argentina. All complexes were classified to the correct mine shape.



**Fig. 5.** First two functions from a discriminant analysis based on a combination of mine shape and contrast, using the abundance of specialized (one to four hosts) and generalist (17 or more hosts) parasitoids for 28 agromyzid hosts in Central Argentina. Highly contrasting mines were represented by open symbols, cryptically coloured mines by solid symbols. Linear, linear-blotch and blotch mines were represented by diamonds, triangles and circles, respectively. All complexes were correctly classified.

On the other hand, specialization probably involves a trade-off between efficiency in resource use and the number of resources that can be used; for parasitoids, an advantage of increased specialization might be a more efficient host location (Godfray 1994), in this case allowing them to find less conspicuous mine types and the hosts within them. Both mine apparency and host escape behaviour (Djemai *et al.* 2000) could explain the higher number of specialist parasitoid species associated to blotch and cryptically coloured mines.

Heterogeneity in parasitoid diversity and impact can also be determined by the degree of host-refuge availability (Hochberg & Hawkins 1992); such refuges may involve spatial protection, physiological traits or concealment within plant structures (Holt, Hochberg & Barfield 1999). The potential for refuges from parasitoid attack is particularly high for concealed hosts

where it can be associated, for example, to gall diameter (Price 1988).

In the case of leafminers, the degree of refuge afforded by the mine could be based on its appearance. Given their extremely limited escape possibilities (but see Connor & Cargain 1994; Djemai *et al.* 2000), leafminer defence must rely on primary mechanisms such as remaining inconspicuous or concealed. The hypothesis of a discoverability gradient for leafminer species, from the very conspicuous linear and highly contrastingly coloured galleries to the least obvious, cryptically coloured blotch mines, is supported by the variations in species number and parasitism rates of generalist and specialist parasitoids in Central Argentina. Specialists decreased and generalists increased in diversity and abundance as hosts became easier to discover. Considering that many other factors could and almost certainly are influencing leafminer parasitic complexes (Hawkins 1994; Salvo 1996), it seems particularly encouraging that nearly half the above-mentioned variability could be explained by the proposed gradient. The observed relationship also indicates that although mine colour by itself is not a strong determinant of parasitoid community structure, it might add an extra dimension to the effect of mine morphology.

The importance of the combination of mine shape and contrast was reinforced by the results of multivariate analyses on specific composition of the parasitic assemblages. Assigning hosts into groups equivalent to those in the proposed discoverability gradient revealed even more significant differences among the associated parasitic assemblages than did their grouping according to mine shape only. Contrast seemed more important in the case of hosts dwelling in blotch mines for at least part of their development (blotch and linear-blotch). Hosts in linear mines supported the most homogeneous parasitic complexes, suggesting that the conspicuousness of the linear shape can override the effect of mine contrast.

We have presented a first approach in understanding how the shape and colour of leaf mines can affect the associated parasitoid assemblages. Comparative studies can be biased by a possible lack of independence among data points (Godfray 1994), and in our particular case phylogenetic effects could not be completely ruled out when considering mine shape. Species producing each mine shape tended to concentrate in a particular genus. However, it must be noticed that linear, linear-blotch and blotch mines were all represented by species belonging to at least four genera. Therefore, we believe the results presented here reflect more than a mere association between host phylogeny and parasitoid assemblages.

Our study suggests that the effects of mine appearance, previously known only for particular host-parasitoid associations, can be important factors shaping parasitoid communities and determining a differential impact of specialist and generalist parasitoids. Further developments of these results could be achieved by

employing a more data-extensive approach such as meta-analysis, and by additional studies and tests of our predictions in other systems.

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## Appendix I

Parasitic species associated to agromyzid leafminers in Córdoba, Argentina, indicating degree of specialization and taxonomic family

Host-range interval	Parasitoid species*	Taxonomic family	
1 host species (specialists)	<i>Lorenzopius calycomyzae</i>	Braconidae	
	<i>Phaedrotoma denticlypealis</i>	Braconidae	
	<i>Ph. riberoensis</i>	Braconidae	
	<i>Phaedrotoma</i> sp. A	Braconidae	
	<i>Phaedrotoma</i> sp. C	Braconidae	
	<i>Pteromalidae</i> sp. A	Pteromalidae	
	<i>Chrysonotomyia</i> sp. B	Eulophidae	
2–4 host species	<i>Chrysonotomyia</i> sp. C	Eulophidae	
	<i>Diaulinopsis</i> sp.	Eulophidae	
	<i>Halticoptera</i> sp. B	Pteromalidae	
	<i>Herbertia</i> sp.	Pteromalidae	
	<i>Phaedrotoma angicypealis</i>	Braconidae	
	<i>Ph. brevimarginalis</i>	Braconidae	
	<i>Phaedrotoma</i> sp. B	Braconidae	
	<i>Phaedrotoma</i> sp. F	Braconidae	
	<i>Tetrastichus</i> sp.	Eulophidae	
	<i>Thinodytes</i> sp. E	Pteromalidae	
	<i>Zaeucoila</i> sp. B	Eucoilidae	
	5–8 host species	<i>Cirrospilus</i> sp.	Eulophidae
		<i>Elachertinae</i> sp. A	Eulophidae
<i>Elachertinae</i> sp. B		Eulophidae	
<i>Thinodytes</i> sp. B		Pteromalidae	
<i>Thinodytes</i> sp. C		Pteromalidae	
<i>Zaeucoila</i> sp. A		Eucoilidae	
<i>Zaeucoila</i> sp. C		Eucoilidae	
9–16 host species	<i>Chrysocharis caribea</i> Boucek	Eulophidae	
	<i>Chrysocharis</i> sp.	Eulophidae	
	<i>Phaedrotoma luteoclypealis</i>	Braconidae	
	<i>Thinodytes</i> sp. A	Pteromalidae	
	<i>Agrostocynips clavatus</i>	Eucoilidae	
17–36 host species (generalists)	<i>Chrysocharis flacilla</i>	Eulophidae	
	<i>Ch. vonones</i>	Eulophidae	
	<i>Chrysonotomyia</i> sp. A	Eulophidae	
	<i>Diglyphus websteri</i>	Eulophidae	
	<i>Halticoptera helioponi</i>	Pteromalidae	
	<i>Poacrias xenodice</i>	Eulophidae	
	<i>Phaedrotoma mesoclypealis</i>	Braconidae	
	<i>Ph. scabriventris</i>	Braconidae	
	<i>Proacrias thysanoides</i>	Eulophidae	

\*Authorities in Salvo & Valladares (1998).

## Appendix 2

Agromyzid species and levels of studied factors (mine attributes) used in data analysis. L = linear mines, LB = linear-blotch mines, B = blotch mines, HC = highly contrasting mines, CC = cryptically coloured mines

Agromyzid species	Shape	Contrast
<i>Agromyza</i> Fallen sp.	L	HC
<i>Amauromyza maculosa</i> (Malloch 1913)	B	CC
<i>Calycomyza brewerae</i> Valladares 1981	LB	HC
<i>C. cruciata</i> Valladares 1982	B	HC
<i>C. lantanae</i> Frick 1956	B	CC
<i>C. malvae</i> (Burgess 1880)	L	HC
<i>C. mikaniae</i> Spencer 1973	B	HC
<i>C. verbenivora</i> Spencer 1963	LB	CC
<i>Chromatomyia platensis</i> (Brèthes 1923)	L	CC
<i>Haplopeodes lycivorus</i> Valladares 1998	LB	CC
<i>Haplopeodes</i> Steyskal sp. A	LB	HC
<i>Haplopeodes</i> Steyskal sp. B	LB	HC
<i>Haplopeodes</i> Steyskal sp. C	LB	CC
<i>Japanagromyza polygoni</i> Spencer 1973	B	CC
<i>Liriomyza brassicae</i> (Riley 1884)	L	HC
<i>L. caesalpiniae</i> Valladares 1982	LB	CC
<i>L. commelinae</i> (Frost 1931)	L	HC
<i>L. huidobrensis</i> (Blanchard 1926)	L	HC
<i>L. near sabaziae</i> Spencer 1973	L	HC
<i>L. sativae</i> Blanchard 1938	LB	HC
<i>L. schmidti</i> (Aldrich 1929)	L	HC
<i>L. spencerella</i> Valladares 1982	L	HC
<i>Liriomyza</i> Mik sp. A	L	CC
<i>Liriomyza</i> Mik sp. B	L	HC
<i>Phytoliriomyza jacarandae</i> Steyskal & Spencer 1978	LB	CC
<i>Phytomyza crassisseta</i> Zetterstedt 1935	L	HC
<i>P. pampeana</i> Blanchard 1954	B	CC
<i>P. williamsoni</i> Blanchard 1938	L	HC

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