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Hiding among holes: mechanisms underlying the evolution of masquerade in flea beetles (Chrysomelidae)

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Abstract. 1. The herbivorous flea beetles (Chrysomelidae: Alticini) have evolved a masquerading strategy by hiding among their own feeding damage. Similarities between beetle bodies and their feeding damage may act as a defence that reduces detection by visual predators.

2. We analyse the evolutionary origin of flea beetles' camouflage using the French fauna (284 species) as a case study. We assess two competing hypotheses: (H1) the type of leaf tissue is the primary determinant of the colour and size of beetle species feeding on them, because each type of leaf tissue may have a tendency to result in a particular type of damage or, (H2) the morphological characteristics of the beetle explain the damage pattern, because beetle species evolve strategies to produce feeding damage that matches its own characteristics.

3. We assessed bipartite networks of feeding interactions (beetle species and host plants). Beetle-host interactions were non-randomly distributed and highly modular, with more than 25% of the network modules showing beetle colour and size distributions significantly different from null expectations.

4. Our results suggest that the evolutionary origin of flea beetles masquerading can be partially explained by both hypotheses. Some host plants seem to restrict feeding damage appearance on their leaves, favouring the survival of specific beetles with matching colour and size (H1). However, in most plant taxa, it is suggested, the existence of beetle-associated constraints exert a selective pressure for the beetle to damage leaves in a particular way, similar to its own colour and size (H2).

Key words. Adaptive evolution, Alticini, camouflage, host plant specialisation, mimicry.

Introduction

Camouflage is a term used to describe all forms of concealment, including prevention of detection and recognition, with the strategies involved in nature being diverse and sometimes extraordinary (Stevens & Merilaita, 2009). Masquerading is a particular type of camouflage, in which organisms resemble inanimate objects, usually inedible that are found in their same habitat. Individuals using this defensive strategy are assumed to reduce predation or gain access to prey by being misidentified as either inedible objects by their predators or as innocuous objects by their prey (Skelhorn *et al.*, 2010a, 2010b). To achieve this, some animals are able to flexibly change their own body characteristics depending on the environment. As an example, the peppered moth (*Biston betularia*) larvae have evolved a twig-mimicking masquerade, changing their colour in response to colour cues from the twigs in the immediate surroundings (Eacock *et al.*, 2019); or the cuttlefish (*Sepia officinalis*) that among other camouflage tactics, may choose to resemble an inanimate object such as seaweed or a rock by controlling its skin colour, contrast, pattern and physical texture

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Fig. 1. *Longitarsus tabidus* on the surface of a leaf of its host plant *Verbascum* sp. (Scrophulariaceae). Russia, Smolensk Oblast, Urga river.

(Buresch *et al.*, 2011). Similarly, some species modify their environment to the same end. For instance, the orb web spider (*Cyclosa ginnaga*) adds a white discoid-shaped silk decoration to its web and positions itself upon it, so that when viewed together, the spider and decoration look remarkably like a bird dropping (Liu *et al.*, 2014; Skelhorn, 2015). At the opposite end, some species have developed persistent body characteristics or behaviours to conceal themselves, like many caterpillars and stick insects with body shapes resembling twigs or narrow stems (Quicke, 2017). This phenomenon has also been described in flea beetles (Coleoptera, Chrysomelidae, Galerucinae, Alticini). These beetles feed on the leaves of their host plants, producing holes that resemble the shape and colour of the beetles' own bodies (Figs. 1 and 2). Thus, their bodies are surrounded by holes that, in two-dimensions, look like the body of the beetle, making it difficult to be identified by predators (Konstantinov *et al.*, 2018).

Alticini beetles are the most diverse tribe of leaf beetles (Chrysomelidae), with approximately 590 genera and about 9900 described species (Konstantinov, 1994; Konstanvinov, 2016). These beetles are highly specialised phytophagous insects (Biondi *et al.*, 2013) whose adults feed above ground on various parts of higher plants, mostly on leaves, but also on non-woody stems, roots and rarely flowers (Konstantinov & Vandenberg, 1996). Hole-making in a leaf surface is a common feeding pattern in adult flea beetles in contrast to other taxa that feed on leaf margins or the lower side of the leaves (Ren *et al.*, 2018). Feeding patterns are species-specific, with

Fig. 2. *Phyllotreta atra* on the surface of a leaf of its host plant *Rorippa armoracioides* (Brassicaceae). Russia, Smolensk Oblast, Urga river.

the resulting holes varying in size and colouration, which can be roughly classified as light or dark. The holes have light tones when the beetle feeds scraping the upper epidermis and only a part of the mesophyll, thus unveiling the light-coloured matrix. Dark tones are produced when the beetle makes holes that penetrate the entire leaf surface, allowing the shadowed background behind them to be observed (Konstantinov *et al.*, 2018). As for the extent of the hole, it is dependent on the volume of plant material ingested by the individual.

Flea beetles are prey to insectivorous birds, for example swifts (*Apus apus*) that are documented to prey on *Phyllotreta atra* (Lack & Owen, 1955), *P. nemorum* (Lack & Owen, 1955; Owen, 1955), *P. nigripes* (Campbell, 1994), *P. vittula* (Owen, 1955) and *Aphthona euphorbiae* (Owen, 1955); or sand martins (*Riparia riparia*), known to prey on *P. nigripes* (Whitehead, 1991). Other visually-oriented generalist predators that are documented to prey on flea beetles are hunting and ambush spiders from the families Anyphaenidae and Salticidae (Riechert & Bishop, 1990). Notably, flea beetles possess a well-known antipredator mechanism: a remarkable jumping ability for long distances at astonishing velocities, allowing sudden disappearance (Maulik, 1929; Chapman, 1998; Nadein & Betz, 2016; Ruan *et al.*, 2020). However, this mechanism would not explain why birds seem to be more efficient predators when beetles are on the ground rather than on their host plants (Lindroth, 1971). Instead, the protection against predators offered by host plants may be related to the existence of an additional defensive strategy that Konstantinov *et al.* (2018) attributed to the mimicry offered by their feeding damage. This former study showed that body colour in flea beetles is related to feeding damage colour (dark or light), and beetle size is correlated with feeding hole extension. Thus, it was hypothesised that adult flea beetles have evolved a close resemblance between their body characteristics and their own feeding damage (Konstantinov *et al.*, 2018). This masquerading mechanism would function as a primary defence reducing predation risk

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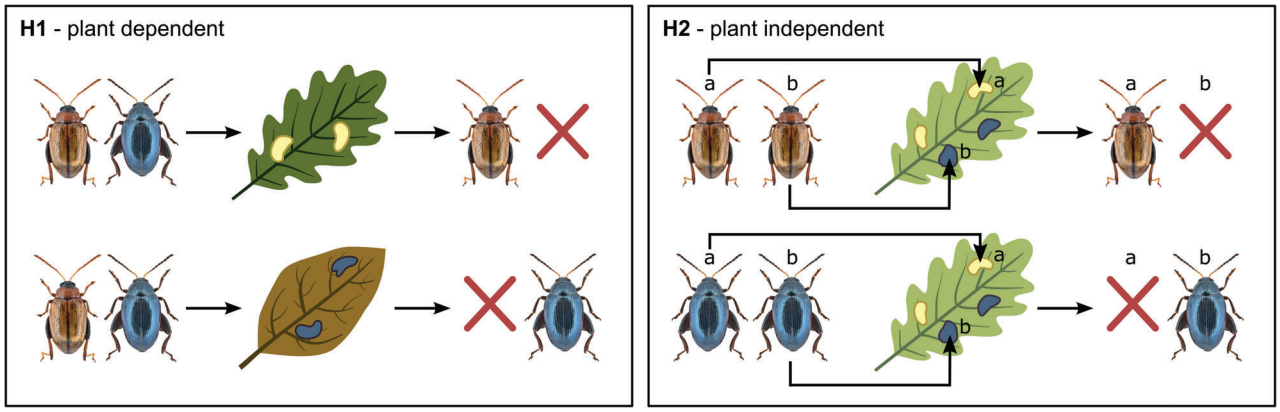

Fig. 3. Alternative hypotheses to explain the evolutionary origin of the resemblance between flea beetles and their feeding damage. Under Hypothesis 1 (H1, plant-dependent) feeding damage depends on the characteristics of the plant species' leaf tissues, which would select beetles matching the plant-determined feeding hole colour and size. Under Hypothesis 2 (H2, plant-independent), beetle morphological characteristics could exert selective pressure on its own feeding pattern to maximise camouflage and, therefore, increase its survival rate and fitness. Therefore, in light coloured species, individuals producing light feeding damage (marked with a) would be selected, while in dark coloured species, individuals producing dark feeding damage (b) would be selected.

by visually oriented predators, such as insectivorous birds and spiders, which would misclassify the beetles as feeding damage rather than viable prey (although empirical experiments have not been conducted thus far). Reducing the ability of predators to detect flea beetles would increase detection time and provide beetles with the opportunity to escape using their remarkably fast and characteristic jumping ability as a secondary defence (Dimitrova & Merilaita, 2009; Xiao & Cuthill, 2016; Konstantinov *et al.*, 2018).

Two hypotheses (based on two contrasting selective processes) here proposed to explain the evolutionary origin of the resemblance between flea beetles and the damage they cause when feeding on leaves (Fig. 3). Under Hypothesis 1 (H1, plant-dependent) leaf tissues of host plants could be prone to be damaged in a particular way (i.e. superficial damages [light holes] or deep holes [dark holes]) due to the characteristics of their leaf tissues, and this would favour the survival of beetles matching the plant-determined feeding hole colour and size. If feeding holes aspect is conditioned by the characteristics of the plant, then only the beetles resembling the holes that occur in each plant would be benefited from masquerading. According to this hypothesis, an association is expected between the taxon of the host plant and specific morphological traits of the beetles (colour and size of the beetles selected by the type of plant). Under Hypothesis 2 (H2, plant-independent), beetle morphological characteristics could exert selective pressure on its own feeding pattern to maximise camouflage and, therefore, increase its survival rate and fitness. According to this hypothesis, no association is expected between beetle traits and the plant taxon because beetle species with different traits (light and dark beetles, for example) could feed on the same plant by using disparate feeding patterns. On the contrary, an association between beetle size and beetle colour is expected under this hypothesis, as beetle size (head size in particular) would condition its ability to cut complete holes across the leaf tissue.

Both hypotheses H1 and H2 are based on the assumption that the masquerading exists, as reported by Konstantinov *et al.* (2018). We first re-assess this assumption by examining the available direct observations ($N = 136$) of feeding damage colour for which beetle colour, beetle identity and host plant family are available. This limited dataset provides direct evidence about the correlation between beetle and feeding damage colour (i.e. the existence of masquerading). Second, we aim to test if empirical observations across a whole national fauna, French flea beetles as a case study (Doguet, 1994), are in accordance with the predictions of either Hypothesis 1 (plant-dependent) or Hypothesis 2 (plant-independent). In other words, we aim to assess whether the type of leaf tissues have selected the colour and size of beetle species because of its propensity to being damaged in a particular way or, alternatively, whether morphological characteristics of the beetle have selected the damage pattern that it produces.

Material and methods

First, we re-assessed the empirical observations reported by Konstantinov *et al.* (2018) with additional observations made after 2018, which makes it the most complete dataset available to spring 2021 (Table S1). This consists of a series of field observations ($N = 136$) reporting flea beetle genus, beetle body colour, feeding damage colour and host plant family. Observations were conducted from 2011 to 2021 on all continents except Antarctica and Australia (12 countries in total) in a variety of habitats from tropical rain forests to deserts and from low land river valleys to alpine meadows. Flea beetles from 54 genera (about 10% of World fauna) were observed. For many genera, more than one species was documented. Some, more common, species were observed during multiple years on same plant species in a variety of habitats across species range. We used generalised linear models to assess the relationship between feeding damage

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colour (binomial factor, dark vs. light), and two potential predictors, beetle body colour (binomial factor, dark vs. light) and plant family (multinomial factor). Models were fit with the glm() function of the 'stats' package (*v3.6.1*; R Core Team, 2019) for R (R Core Team, 2019), using binomial error distribution and logit link.

The French fauna of flea beetles (Chrysomelidae: Alticini) was used as a case study because of its completeness regarding both taxonomic knowledge and information on host plant associations. Data have been collected from Doguet (1994) with a total of 284 flea beetle species belonging to 27 genera, including their coloration and body size, as well as their respective host plants (families and genera). Beetle colour was categorised as (i) 'light', from yellowish to light brown tones; or (ii) 'dark', including black, blue, red and metallic green colours. Bicolour patterns were classified according to the dominant colour. On the other hand, beetle size was computed as the mean between maximum and minimum lengths (as reported in Doguet, 1994) and categorised as 'small' (≤2.50 mm), 'medium' (2.50–3.75 mm) or 'large' (>3.75 mm). This dataset is available in Table S2.

To preliminarily assess whether the frequency of an association between a given host plant (at family or genus level) and a specific beetle trait (colour or size) is non-random, a χ^2 test was applied. The dependency relationship between the following pairs of qualitative variables was studied: host plant family and associated beetle colour, plant family and beetle size, plant genus and beetle colour, plant genus and beetle size. In addition, given the high proportion of host plants with only one beetle species (considered singletons), the same analysis was also conducted on a subset with no singletons to avoid potential statistical biases.

To evaluate the structure of interactions among the complete French flea beetle fauna, we built a host plant versus beetle bipartite network (Dormann & Strauss, 2014). We decided to compute two different networks: one with plant families and the other with plant genera, separately. Many networks are found to be naturally divided into modules, that is, subgraphs of data from both levels whose vertices are more likely to be connected to one another than to the vertices outside the subgraph (Newman, 2006; Barber, 2007). The prevalence of modules in the network can be measured, so that modularity (Q) is an estimate of the extent, relative to a null model network, to which interactions are likely to appear within modules instead of between modules. Using the 'BIPARTITE' package (Dormann *et al.*, 2009) for R (R Core Team, 2019), we computed Newman's modularity of each of the networks returning the most modular result. To obtain the significance of the observed value, we compared it with null models of 100 randomised network replicates of the same characteristics. The obtained values were corrected by null model expectations and standardised to z-scores ($z_Q = \frac{Q_{\text{observed}} - \overline{Q}_{\text{null}}}{\sigma Q_{\text{null}}}$). Since z-scores follow a standard normal distribution, values above 2 are considered significantly modular (Dormann & Strauss, 2014).

To assess whether host plants in a module were predominantly associated to a particular colour (dark or light) or body size, we compared beetle colour and size within modules, measured as the percentage of dark beetles and standard deviation of body size, respectively, with null expectations. The percentage of dark beetles in each module was compared to 100 000 null models with the same number of beetle species, but randomly selected from within the entire dataset. Similarly, beetle size standard deviation in each module was also compared with the standard deviation obtained in 100 000 null models consisting of the same number of beetle species but again randomly selected from within the entire dataset. Comparison P-values were calculated for each variable (colour and size) and taxonomic level (plant family and genus), considering statistically significant values of $P < 0.05$. Modules with less than 10 species were excluded from this analysis to avoid small sample sizes, which may lead to bias. Statistically significant differences between observed values and null model simulations would support the hypothesis that beetles with a certain characteristic, colour or body size, are associated with certain host plants (H1); while the absence of significant differences would support a random distribution of beetle colours and sizes in host plants (H2). In addition, an association between beetle colour and size would provide evidence supporting H2. Thus, the relationship between the studied morphological variables (colour and body size) was assessed to discern whether a specific tone colour is associated to a certain size or if, contrarily, the size of the beetle is independent of the colour it presents. For this, a one-way analysis of variance test was applied using the 'stats' package (v3.6.1; R Core Team, 2019) for R (R Core Team, 2019). We assessed the effect of the colour factor, with two levels (light and dark), on mean body size as the response variable.

Results

Using the dataset of empirical observations on feeding damage coloration, we found that feeding damage colour was tightly associated both to beetle colour (McFadden pseudo-$r^2 = 0.43$, $P < 0.0001$) and to host plant family (McFadden pseudo-$r^2 = 0.53$, $P < 0.0001$). Notably, beetle colour was also tightly associated to plant family (McFadden pseudo-$r^2 = 0.603$, $P < 0.0001$), suggesting that the link between feeding damage colour and plant family could arise from the beetle's host specificity and/or the phylogenetic autocorrelation, rather than from the morphological characteristics of plant leaves. To remove this potential phylogenetic autocorrelation effect, we performed an additional analysis on a limited subset of observations within a single genus (*Longitarsus*, the only one with a number of observations large enough to allow statistical testing). For this subset, damage colour was still tightly correlated to body colour (McFadden pseudo-$r^2 = 0.66$, $P < 0.0001$) but not to host plant family (McFadden pseudo-$r^2 = 0.16$, $P = 0.1340$). This suggests that, at least for the genus *Longitarsus*, the association between feeding damage and body colour has arisen independently of the host plant family (H2).

To assess how generalisable this result is, the French alticine fauna was analysed. The French flea beetles were recorded to feed on 59 host plant families and 305 plant genera (Doguet, 1994). The distribution of beetles among host plants is not uniform, so that the 10 plant families with a higher number of interactions (Brassicaceae, Asteraceae, Lamiaceae, Boraginaceae, Euphorbiaceae, Plantaginaceae, Poaceae, Cyperaceae,

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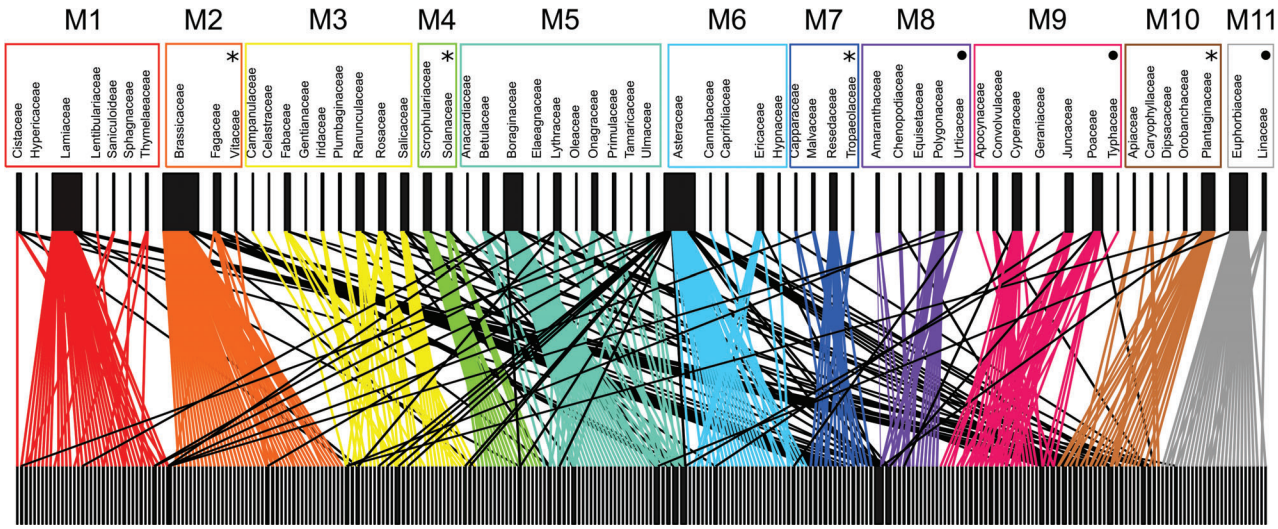

Fig. 4. Bipartite network of beetle species (lower level) and host plant families (upper level). Eleven modules were identified based on Newman's modularity. Plant-beetle interactions within a module are represented by the same colour. Interactions not belonging to any module are coloured in black. Modules marked by * or • showed colour or size distributions significantly different from null expectations, respectively.

Polygonaceae and Juncaceae, by descending order) are associated with 75.0% of the studied beetle species (213 out of 284). Moreover, the first five aforementioned plant families are associated with 63.7% of beetle species (181 out of 284). Many flea beetles are monophagous insects and this host specificity is represented as singletons in our data (i.e. a beetle species associated with just one family or genus of host plant). Specificity is much higher at the plant family level, with 69.4% of beetle species (197 out of 284) associated with just one plant family, in comparison to the 34.5% of the beetle species (98 out of 284) associated with just one plant genus. In contrast, some beetle species can be considered generalists, as 4.2% of French flea beetle species (12 out of 284) were associated with four or more different plant families, and 33.8% of beetle species (96 out of 284) were associated with four or more plant genera.

Beetle species were classified accordingly to their body colour and size. A total of 70.0% (199 out of 284) of the species showed dark colouration (black 40.0%, blue 17.0%, green 8.0% and red 5.0%), and the other 30.0% (85 out of 284) presented light colours (light brown 8.0% and yellow 21.0%). Considering body size, 62.0% (176 out of 284) of the species were less or equal than 2.50 mm long, and thus included in the 'small' range, 31.3% (89 out of 284) were 'medium' size, between 2.50 and 3.75 mm and the other 6.7% (19 out of 284) presented bodies bigger than 3.75 mm, the 'large' beetle species.

According to the results of the χ^2 test, the studied beetle traits (colour as dark or light; size as small, medium or large) are non-randomly associated to host plant families (beetle colour: $\chi^2 = 133.30$, $P < 0.001$; beetle size: $\chi^2 = 251.64$, $P < 0.001$) and genera (beetle colour: $\chi^2 = 550.06$, $P < 0.001$; beetle size: $\chi^2 = 913.31$, $P < 0.001$). To avoid potential statistical biases, χ^2 tests were repeated omitting singletons, however, the obtained P-values were still significant ($P < 0.001$ in all cases), so they were not excluded from the following analyses.

Modularity in the beetle-plant bipartite network was high both at family ($Q = 0.686$, $z = 18.15$, $P < 0.001$) and plant genus levels ($Q = 0.716$, $z = 63.74$, $P < 0.001$). At the plant family level, 11 modules were identified, each one containing from 13 to 42 beetle species (mean = 25.8) (Fig. 4); while at the plant genus level, 17 modules were considered, each one containing from 1 to 48 beetle species (mean = 16.2) (Fig. 5). The proportion of dark versus light-coloured beetle species were significantly different ($P < 0.05$ and > 0.95) from null expectations in 4 out of 11 modules (36.0%) in the beetle-plant family network (Table 1), and in 5 out of 17 modules (29.4%) in the beetle-plant genus network (Table 2). The variation (standard deviation) in body size across beetle species belonging to a given module was significantly smaller than null expectations in 3 out of 11 modules (27.0%) in the beetle-plant family network (Table 1), and in 4 out of 17 modules (23.5%) in the beetle-plant genus network (Table 2). Finally, dark beetles were significantly larger than light beetles (2.68 ± 0.82 vs. 2.05 ± 0.49 mm, respectively, $F_{1,282} = 43.4$, $P < 0.001$).

Discussion

This study suggests that the evolutionary origin of the newly discovered masquerade decoy by Konstantinov *et al.* (2018) can be partially explained by two alternative hypotheses. While some plant taxa are preponderantly associated with a subset of beetle species that have a specific body morphology (based on their colour and size), most plant taxa in our data seem to be associated with morphologically different beetle species. In other words, the beetle colour and size do not differ from what we would expect if they were randomly distributed among plant taxa. In the first case, we suggest that host plants might condition feeding damage appearance on their leaves, favouring the survival of specific beetles with matching colour and size

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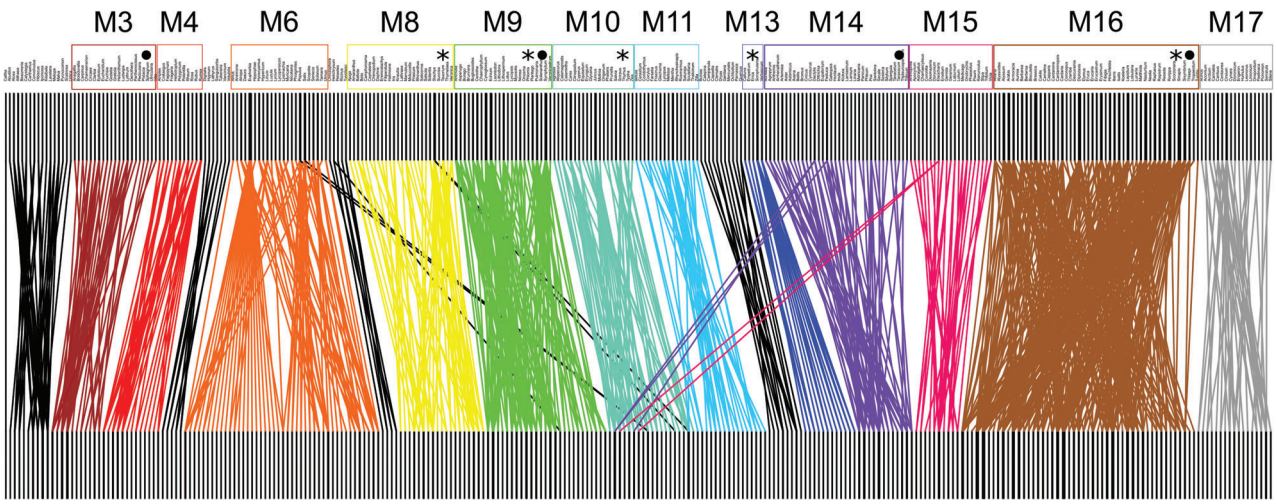

Fig. 5. Bipartite network of beetle species (lower level) and host plant genera (upper level). Seventeen modules were identified based on Newman's modularity. Plant-beetle interactions within a module are represented by the same colour. Interactions not belonging to any module or from non-significant modules are coloured in black. Modules marked by * or • showed colour or size distributions significantly different from null expectations, respectively.

Table 1. Comparison of network modules with null model expectations attending to beetles body colour and size in relation with plant families.

		Colour		Size	
Module	n^*	dark (%)†	*P*-value	SD‡	*P*-value
1	37	64.86%	0.1934	0.6497	0.0864
2	42	95.24%	**0.9999**	0.6567	0.0790
3	30	73.33%	0.6210	0.8443	0.7092
4	16	37.50%	**0.0012**	0.6925	0.3034
5	31	67.74%	0.3358	1.0750	0.9962
6	29	62.07%	0.1344	0.8712	0.7847
7	14	92.86%	**0.9670**	0.8614	0.6987
8	13	76.92%	0.6895	0.4759	**0.0494**
9	25	64.00%	0.2126	0.4299	**0.0023**
10	21	47.62%	**0.0074**	0.6961	0.2729
11	26	80.77%	0.8748	0.5303	**0.0191**

In bold *P*-values showing significant results ($P < 0.05$ and >0.95 for colour; $P < 0.05$ for size).
*Number of beetle species in the module.
†Percent of dark beetles in the module.
‡Average standard deviation of the beetles within each module for the module size mean.

Table 2. Comparison of network modules with null model expectations attending to beetles body colour and size in relation with plant genera.

		Colour		Size	
Module	n^*	dark (%)†	*P*-value	SD‡	*P*-value
1	1	–	–	–	–
2	9	–	–	–	–
3	11	90.91%	0.9316	1.2550	0.9938
4	13	76.92%	0.6886	0.3689	**0.0123**
5	5	–	–	–	–
6	44	79.55%	0.9163	0.7498	0.3444
7	4	–	–	–	–
8	19	52.63%	**0.0375**	0.6263	0.1476
9	26	50.00%	**0.0071**	0.5095	**0.0121**
10	20	90.00%	**0.9735**	0.6077	0.1131
11	16	56.25%	0.0926	1.0020	0.9242
12	8	–	–	–	–
13	12	25.00%	**0.0002**	0.9363	0.8176
14	12	50.00%	0.0511	0.3920	**0.0216**
15	10	50.00%	0.0713	0.7128	0.4021
16	48	95.83%	**1.0000**	0.6003	**0.0139**
17	17	70.59%	0.4930	0.7485	0.4292

The analyses for the modules composed of less than 10 beetle species are not presented in this table. In bold *P*-values showing significant results ($P < 0.05$ and >0.95 for colour; $P < 0.05$ for size).
*Number of beetle species in the module.
†Percent of dark beetles in the module.
‡Average standard deviation of the beetles within each module for the module size mean.

(H1). However, in most plant taxa the random distribution of beetle colour and size suggests the existence of beetle-associated constraints that exert a selective pressure for the beetle to damage leaves in a particular way, similar to its own colour and size (H2). In both situations, visually oriented predators take a role in selecting those combinations of beetle morphology-hole appearance that maximise camouflage effectiveness.

Our Hypothesis 1 (plant-dependent) assumes the existence of morphological and/or physiological plant-associated constraints capable of determining beetles feeding habits. Furthermore, these characteristics must be plant taxon-specific. To analyse, which plant characteristics conduct the type of feeding damage produced is not an objective of this study, but there are some examples that suggest that these mechanisms exist. For example Bodnaryk (1992a) identified two distinctive leaf-feeding patterns (edge feeding and randomly distributed

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feeding) done by the flea beetle *Phyllotreta cruciferae* on Brassicaceae host plants. Several experimental approaches were undertaken in an attempt to understand the basis of this feeding behaviour, revealing a concordance between the feeding pattern used by the beetle and the selected plant species. A later article (Bodnaryk, 1992b) showed that leaf epicuticular wax was determining the feeding rate and pattern. Despite this, there is no evidence on how general this phenomenon is, beyond the presented example that encompasses a single alticine species. The question thus remains open for future research.

Next, Hypothesis 2 (plant-independent) does not consider any plant-associated constraints but it assumes the existence of cognitive, morphological and/or physiological beetle-associated constraints able to determine their own feeding damage pattern. For instance, the depth of the hole would be explained by the size of the beetle under H2. Previous studies have found that extremely small flea beetles, *Aulacothorax syzygium* and *Aulacothorax terminalia* (length 1.2–1.6 mm), are incapable of cutting holes in the leaf lamina but rather produce feeding trenches on the adaxial surface of leaves of *Terminalia* and *Syzygium* (Prathapan *et al.*, 2013), while larger beetles, like some members of the Curculionidae family (length 2.2–4.5 mm), are capable of making deep holes on the same plant species. Further research should assess the generality of this observation, but it suggests that body size can constrain the depth that the feeding damage can reach in the leaf tissue. The existence of such a physical constraint in the beetles would lead to an association between colour and size, as masquerading of large beetles would only work if they were dark. This association is indeed present in the French fauna dataset, with dark species tending to be larger than light species, which is in accordance with the idea that beetle size acts as a constraint to hole depth, and hence to beetle colour via natural selection. This restriction, according to our H2, would generate a selective pressure on the colour tone of the beetle. Regarding hole size, it was found that two factors of the Alticini beetle *Altica cirsicola* anatomy directly determine the feeding pattern: (i) the flexibility of the head-prothorax, and (ii) the volume of the beetle digestive system. The head-prothorax mobility is responsible for hole width, while the foregut volume constrains meal consumption, which in turn limits the hole extension (Ren *et al.*, 2018). To sum up, beetle size, head-prothorax mobility and digestive system volume are beetle-associated constraints demonstrated to determine feeding hole features, which provide mechanistic evidence supporting the plant-independent hypothesis behind masquerading.

In both situations, variability must exist, in terms of fitness and survival of individuals, between those beetles with an appearance similar to the feeding holes they make, and those that have not developed masquerade. If masquerade did not provide a selective advantage, via reduced predation, similarities between body morphology and feeding damage may occur across some individuals in populations by chance, but it would not become a widespread trait among flea beetles over evolutionary time, as seems to have occurred. Visually oriented predators, such as insectivorous birds, have been proposed as those responsible for the intense selection that has driven flea beetles to evolve this unique variant of the masquerade strategy (Konstantinov *et al.*, 2018; Ren *et al.*, 2018). Still, there are no empirical data that demonstrate that birds suffer reduced search efficiency when confronting beetles with this type of camouflage. However, it has been concluded, based on computer predator simulations that visual searches using humans as test subjects become more inefficient as feeding hole number increases and as hole size approaches beetle body size (Ren *et al.*, 2018).

Nonetheless, these results must be interpreted with caution. Flea beetles are distributed worldwide (Konstantinov & Vandenberg, 1996) and, although our field observation dataset matches this scale, we used a more spatially restricted compilation of diverse observations (French fauna) for the bipartite network analysis. Furthermore, even in one of the best-studied faunas, such as the French, information on beetle host plants is incomplete, making it difficult to discern to what extent computed modules are affected by geographic biases rather than by real plant/host associations derived from selective pressures. It could be argued, however, that the French fauna is one of the best datasets we can use to analyse host plant interactions. Even if some new host plants could be discovered in the future, the associations reported in the published monograph must represent a robust picture of the trophic relationships of French alticines, as it compiles observations by multiple taxonomists and naturalists that have literally occurred through hundreds of years. In addition, some methodological simplifications were necessary in order to formalise the analysis. Regarding beetle body colour, in our dataset, there are numerous species of monochromatic Alticini, but it also includes some species characterised by contrasting patterns of light and dark stripes or spots, which have been assigned to the light–dark category depending on the predominant colour. This simplification could introduce some uncertainty in the analysis because we do not know to what extent these patterns affect the visual perception of predators. However, we can highlight that this represents just 10.2% of our beetle species (29 out of 284), while the other 89.8% (255 out of 284) presents monochromatic colours. Therefore, any uncertainty introduced in the analysis should have a small effect on our results.

Another phenomenon that could influence the results is the presence of the so-called phylogenetic niche conservatism. This is a type of evolutionary inertia, in which the species tend to retain ancestral ecological characteristics, so that close species in the phylogeny would show similar ecological niches (Wiens & Graham, 2005). Under this idea, species that have diverged from a common ancestral species tend to occupy a common ecological space (Price, 1997; Harvey & Rambaut, 2000). The degree of niche conservatism is variable in different clades, from the existence of a very strong phylogenetic inertia to a total absence of a phylogenetic signal in ecological niches. Among Alticini, a high proportion of host plant families are preponderantly associated with beetle species of the same genus, suggesting the existence of some degree of phylogenetic conservatism. Therefore, the masquerading strategy could have been evolved in an ancestral beetle species, later diverging in other species that have maintained the original body morphology and the specificity of host plants, since otherwise, it would diminish their chances of survival. However, to analyse the effect of phylogenetic conservatism, it would be necessary to build the phylogenetic tree of all the Alticini species considered. Such a

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phylogeny is not currently available. Therefore, the phylogenetic signal of the studied morphological traits and the ecological trophic niche (host plants) in Alticini would still be open for analysis in the future.

In conclusion, we have found partial support for both types of restrictions associated with plants (H1) and beetles (H2), which together would have contributed to the appearance of masquerade among Alticini. We do not know how or with what preponderance plants act as protagonists delimiting the feeding pattern in their leaves or beetles themselves restrict the damage they do, but possible mechanisms regarding each hypothesis have been discussed in this study. It is not easy to identify the full range of possible selection pressures. Nevertheless, it is likely that visually-oriented predation was the selective force driving the evolution of feeding hole masquerade favouring the survival of those beetles with matching feeding holes. Overall, feeding damage camouflage that creates a constant hole size and tone colour is an adaptation in the long coevolution with the host plant and natural predators.

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Authors' contributions

AB and CGR conceived the ideas and designed the methodology. YFC collected the data, analysed the data and led the writing of the manuscript. ASK documented field observations. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

All the datasets analysed during the current study are provided as Tables S1 and S2.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1: Dataset of the available field observations of feeding damage by flea beetles.

Table S2: Dataset of the flea beetles (Chrysomelidae: Alticini) French fauna.

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