

Conserved oviposition preferences in alpine leaf beetle populations despite host shifts and isolation

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Abstract. 1. Choosing the plant on which to lay their eggs is the last act of care that most female herbivorous insects bestow upon their offspring. These decisions play a pivotal role in insect–plant interactions, placing host preference under strong selection and contributing to the diversity of phytophagous insects as one of the first traits to adapt to new hosts.

2. This study presents a test of whether extreme isolation and exposure to different host plants can produce intra-specific divergence in oviposition preference in alpine insects. Geographic variation should impose selection to fine-tune host plant ranking and specificity to the plants normally encountered, to avoid wasting time during the very limited reproductive season experienced at high altitudes.

3. Beetles from five populations of *Oreina elongata* differing in host availability were offered three natural hosts: *Cirsium spinosissimum*, *Adenostyles alliariae*, and *Adenostyles glabra*. A novel application of a continuation ratio model (logistic regression) was made to sequential no-choice experiments, combined with quasi-likelihood analysis of multiple-choice experiments.

4. The results show little geographic variation in host plant choice: all populations strongly preferred *Cirsium* in multiple-choice trials, and in no-choice experiments laid around 47% of their remaining eggs during each stage, almost regardless of the host present.

5. Enemy-free space seems to explain the preference for *Cirsium*, but isolation and exposure to different plants has clearly not caused local adaptation in host plant ranking or specificity. Reasons for this conservatism despite divergence in other characteristics are discussed.

Key words. Chrysomelidae, Coleoptera, host plant choice, insect–plant interactions, intra-specific biodiversity, local adaptation, *Oreina elongata*

Introduction

Most aspects of the ecology of herbivorous insects revolve around their host plant, and oviposition preference plays a critical role in these interactions. It determines the exposure of eggs to natural enemies and the first food available to larvae, and changes in host acceptance are often the first step in specialisation (Futuyma & Moreno, 1988) and speciation (Schluter, 2001; Via, 2001; Drès & Mallet, 2002). Oviposition preference is understood to be the product of two components: host plant rank-

ing (the hierarchy of acceptable plants) and specificity (the strength of preference for a favoured host over those that are less favoured) (Wiklund, 1981; Courtney *et al.*, 1989; Singer *et al.*, 1992). Under this scheme, two insect species with the same host ranking can show contrasting generalist and specialist behaviour if they differ in specificity. Both elements of preference are likely to show geographic variation as a product of local adaptation to differences in the hosts available.

Host plant ranking might be expected to evolve to match the hierarchy of plants in their suitability for larval growth, yet preference and performance are often not strongly correlated (Thompson, 1988; Jaenike, 1990; Mayhew, 1997). Females are clearly not simply maximising larval growth rate, and the optimal plant species for oviposition in the wild also depends on

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natural enemies, competitors, adult feeding preferences and plant phenology, all of which can vary geographically (Price *et al.*, 1980; Jeffries & Lawton, 1984; Filchak *et al.*, 2000; Scheirs *et al.*, 2000).

Specificity should likewise evolve to reflect the magnitude of differences in survival on different plants, but intra-specific variation is perhaps most likely to arise from differences in host availability. Specificity is expected to be greater in mixed host populations than in those with only the favoured host present, to ensure effective monophagy (Singer, 1982). Conversely, when the optimal host is rare or absent, selection will favour insects that more readily accept lower ranked hosts, if search time limits female fecundity (Wiklund, 1981; Jaenike, 1990; West & Cunningham, 2002) or if delayed oviposition would compromise larval survival (Singer, 1982). This reduction in specificity might evolve through changes in the thresholds for acceptance of individual plant species, or by increasing the rate at which general motivation to oviposit changes (Courtney *et al.*, 1989; Singer *et al.*, 1992; Gotthard *et al.*, 2004).

Alpine insects seem particularly likely to respond to these selection pressures and to show local adaptation of oviposition preference. In many lowland species, northern European populations were repeatedly eliminated and recolonised throughout the Quaternary Period in response to the advance and retreat of the ice sheets (Hewitt, 1996, 2004; Taberlet *et al.*, 1998). Evidence is accumulating that mountain species, in contrast, survived glacial periods *in situ*, remaining within the Alps in peripheral or central refugia (Schönswetter *et al.*, 2005). This long-term isolation may have promoted intra-specific adaptive divergence, by allowing populations to evolve independently. Alpine species also face an extremely short breeding season, so that time constraints impinge upon their oviposition decisions.

Here geographic variation in oviposition preference is tested in the leaf beetle *Oreina elongata* Suffrian (Coleoptera: Chrysomelidae). Isolated populations of this metallic blue-green beetle exist throughout the Alps and Apennines at or above the treeline (1800–2300 m above sea level), and have different plant species available. Migration between populations is highly improbable because they are separated by inhospitable peaks and valleys, and the beetle is small (body length around 7 mm) and has never been known to fly. This translates into strong genetic differentiation: a survey of 13 populations using microsatellites to calculate F_{ST} (representing the heterozygote deficit due to division into subpopulations) yielded an overall estimate of 0.379 and high pairwise values everywhere (Margarf, 2003). Gene flow therefore represents no impediment to local adaptation (Barton & Whitlock, 1997; Lenormand, 2002).

Oreina elongata is a specialist on Asteraceae, feeding on species of *Adenostyles* (tribe Senecioneae) and on *Cirsium spinosissimum* (Cardueae). Host plant use determines the defence chemistry of the beetles: larvae and adults sequester pyrrolizidine alkaloids from *Adenostyles*, whereas on *Cirsium* they synthesise cardenolides (Dobler *et al.*, 1996). Different populations encounter either only *Cirsium*, only *Adenostyles*, or both in mixed patches (Table 1). Although female choice of host plants will have little influence on larval diet because larvae move repeatedly between plants in mixed populations (Gotthard *et al.*, 2005), it will determine egg loss by predation, as, on the spiny leaves of *Cirsium*, eggs benefit from protection against predators (Ballabeni *et al.*, 2001b). *Cirsium* is therefore expected to be the highest ranked host for beetles that encounter it in nature. Geographic variation in host plant availability should generate variation in host preference. Specificity is likely to be greater in mixed host populations than in those with *Cirsium* alone, to ensure oviposition on the safer host. In contrast, in sites with only *Adenostyles* available, either a demotion of *Cirsium* in the rank order or a reduction in specificity would be expected, because a conserved preference for an absent plant would cause females to delay oviposition. This would have a strong impact on these beetles because at high altitudes their breeding season is extremely limited, with the habitat only free of snow between late June and early September. In the field, females lay eggs singly from early July to mid August (Ballabeni *et al.*, 2001a), and in the laboratory can lay several hundred eggs during a season (unpublished data). However, as eggs take 15–20 days to hatch (Ballabeni *et al.*, 2001b) and larval growth requires ≈ 30 days (Margarf *et al.*, 2003), there is a very limited period in which females can lay eggs that stand a chance of completing development before the winter. Inappropriate choosiness in populations lacking the favoured host would therefore impose high costs. In summary, enemy-free space and geographical variation in host availability are likely to have driven divergence in ranking or specificity between isolated populations of this alpine beetle.

A previous study tested for local adaptation of oviposition preference in *O. elongata* and concluded that populations had not altered their ranking of host plants, but that specificity had declined in a population lacking the most favoured host (Gotthard *et al.*, 2004). However, that work included only three populations (so no replication of populations of each type) and only two host plants (so lacked the natural hosts of some populations). Here beetles from five populations are compared in their responses towards all of their natural hosts (*C. spinosissimum*, *A. alliariae*, and *A. glabra*), using a combination of

Table 1. The five *Oreina elongata* populations from the central and western Alps studied here. CH, Switzerland; F, France; I, Italy; asl, above sea level.

Population	Code	Host plants	Altitude (m asl)	GPS coordinates
Bosco Gurin (CH)	BOG	<i>C. spinosissimum</i>	1835	46°18' 18.5"N 8°27' 46.6"E
Lautaret (F)	LAU	<i>A. glabra</i>	1811	45°00' 47.6"N 6°22' 25.3"E
Mattmark (CH)	MTT	<i>C. spinosissimum</i>	2239	46°01' 38.5"N 7°58' 01.6"E
Nice (F)	NCE	<i>A. glabra</i>	2080	44°00' 04.0"N 7°26' 01.9"E
Petit Saint-Bernard (F/I)	PSB	<i>A. alliariae</i> + <i>C. spinosissimum</i>	2188	45°40' 46.0"N 6°52' 57.3"E

multiple-choice and sequential no-choice tests. This provides a test for ecological differentiation in both ranking and specificity, and mimics the two natural situations: when females encounter an isolated host, and when they have a choice between plants in close proximity. An improved statistical approach shows that, in fact, both ranking and specificity are unchanged in isolated populations of *O. elongata*.

Methods

Experiments were conducted at the Col du Petit Saint-Bernard (Table 1). Soon after the snow had melted, between 20 and 24 June 2002, ≈ 150 beetles were collected from each of the five populations in Table 1. All beetles were initially fed with cuttings of their natural host plant and kept in a refrigerator at 10 °C to limit their egg laying. From 24 June, they were given a mixture of *C. spinosissimum* (s), *A. alliariae* (a), and *A. glabra* (g) to give them an initial experience with each of these plants. Leaves of the last host were collected from Lautaret, France (LAU), whereas other plants were found at Petit Saint-Bernard, French/Italian border (PSB). The subsequent experiments were conducted outside, sheltered from strong winds by a south-east facing building but under natural light and temperature conditions.

Experimental design

No-choice. Starting on 29 June, each female was tested on the three host plants (a, g, s) in succession, with the six possible orders randomly allocated. For each stage, a single cutting of a mature host plant leaf (of about 40 cm²) was placed on wet filter paper in a 20-cL plastic cup with a perforated lid. Females were left to oviposit for 48 h before all eggs laid on the plant and cup were removed and counted, and the host plant replaced with a cutting of the next plant in sequence.

From some sites, only a few beetles could be collected, and, after exclusion of individuals that laid fewer than five eggs (most of which laid zero), the total number of females varied among populations: 38 for Bosco Gurin, Switzerland (BOG), 48 for LAU, 72 for Mattmark, Switzerland (MTT), 32 for Nice, France (NCE), and 80 for PSB.

Multiple-choice. On 7 July each female was given 65 cm² cuttings of the three host plants (a, g, s) and a non-host that is common in alpine ecosystems, *Rumex alpinus* (r). The cuttings were arranged at random, with no physical contact, in a square plastic box (dimensions 30 × 30 × 10 cm), with a layer of wet sand to maintain humidity. Fifteen females of each population were individually assigned to a box at random. They were allowed to oviposit for 36 h before the eggs were counted on each plant and on the plastic box (b).

Statistical treatment

No-choice. This experiment was analysed as a series of sequential choices by each female, where she was assumed to have an initial payload of eggs, and to choose at each stage to

lay a proportion of her remaining eggs based on her current environment [plant (e) and box (b)] and history. A standard model in such cases, the so-called continuation-ratio model (Agresti, 2002), would treat the number of eggs laid at successive stages as a sequence of binomial variables, the first of which is the number of eggs laid in stage 1 ($e_1 + b_1$) out of a total possible number of eggs that could have been laid at this stage ($e_1 + b_1 + e_2 + b_2 + e_3 + b_3$). The same approach was used for stage 2, the response being the number ($e_2 + b_2$) of eggs laid out of the remaining ($e_2 + b_2 + e_3 + b_3$), but as the number of eggs left after stage 3 remained unknown, data for stage 3 cannot be analysed in this way. The eggs laid on the box and plant were combined for this analysis, because, in the confined space of the box, all oviposition is likely to have been motivated by the plant present.

The data for each stage consist of the number of eggs laid, n , out of the possible total number that could be laid, m . These numbers are discrete and may be small, so treating them as normal variables will often be inappropriate. A more suitable analysis of such data is through logistic regression, whereby the number of eggs laid is treated as a binomial random variable, the denominator of which is the total number of eggs available to be laid at that stage and the probability of which depends on factors such as insect population, host plant, host plant at a previous stage of the experiment, if any, and their interactions. Such models have been used in a huge range of applications for over 30 years, but closely related models were first used in biology in the 1930s (Bliss, 1935).

Logistic regression does not account for the overdispersion that is commonly seen in practice, as a result of unmodelled sources of variation such as differences among the preferences shown by females from the same population. Such factors increase the variability of the data relative to that expected from a standard model, thus increasing the uncertainty of conclusions and decreasing the power of hypothesis tests. Overdispersion manifests itself through the presence of residual variation that cannot be explained by fitting additional terms in the model, and standard methods have been developed to deal with it (McCullagh & Nelder, 1989; Davison, 2003). Likelihood methods are modified so that the usual χ^2 tests for comparison of nested models through likelihood ratio statistics are replaced by F -tests in which the estimated overdispersion factor appears as a denominator; this factor equals 1.0 when there is no overdispersion, and exceeds this value when the data are more variable than a simple model might suggest. Once a suitable model has been determined, the maximum likelihood estimates of its parameters are used, but to account for the added uncertainty, their standard errors are inflated by the square root of the estimated overdispersion factor (McCullagh, 1991). Of the statistical packages capable of making an appropriate allowance for overdispersion, R was used (Venables & Ripley, 2002; R Development Core Team, 2004; <http://www.r-project.org>).

In a first step, the data from stage 2 were analysed with a logistic regression model including terms for the effects of population, plant and their interaction, plus terms allowing us to test for a carryover effect of the plant to which the female had been exposed in stage 1. Data from stages 1 and 2 were then combined in an overall continuation ratio model, with factors representing

the effects of stage, population, plant, and the population by plant interaction.

Egg placement was analysed in the same way, here comparing the number of eggs laid on the plant (e) out of the total laid (e + b) in each stage, and fitting a model including terms for stage, population, plant, and the population by plant interaction.

Multiple-choice. If females laid each egg independently and with a constant probability for each host plant, then oviposition choices would have a multinomial distribution. This simple model is unlikely to be adequate, because of the overdispersion mentioned above, but the same approach can be used to account for its effects.

The data from the multiple choice experiment were analysed twice, first excluding the eggs laid on the box to test for a preference among the four plant species, and then considering just the binary decision to lay on a plant or on the box. The estimated overdispersion factors of 3.25 and 4.46, respectively, indicate strong overdispersion.

Results

No-choice experiment

Females laid 5–42 eggs in total across the three trials of this experiment [BOG, mean 18.7 (range 5–29); LAU, 21.0 (6–35); MTT, 13.8 (5–29); NCE, 27.7 (8–42); PSB, 19.8 (6–35)]. Although populations differed in the total number of eggs laid ($F_{4,265} = 23.87, P < 0.001$), there was no significant effect of the sequence in which the plants were offered (sequence, $F_{5,260} = 0.96, P = 0.44$, sequence by population interaction $F_{20,240} = 0.96, P = 0.51$). This suggests that females did not mature extra eggs in response to being given a favoured plant early in the experiment, and so the assumption of an initial payload of eggs seems reasonable.

The analysis of stage 2 showed no significant carryover effect of the plant that a female had encountered in stage 1 (plant 1 main effect $F_{2,258} = 0.10, P = 0.90$; plant 1 by population interaction $F_{8,242} = 0.40, P = 0.92$), so only the overall continuation

ratio model is considered. Females laid a relatively constant proportion of their remaining eggs during each stage (Fig. 1). There was no significant effect of population, either as a main effect or in its interaction with plant (Table 2). The effect of host plant was just significant, with on average most eggs laid when *C. spinosissimum* was available, and fewest with *A. alliariae*.

The proportion of eggs laid on the plant rather than on the box during each stage differed highly significantly among populations and among plants, but their interaction was not significant (Fig. 2 and Table 2). Overall averages showed a greater proportion of eggs laid on the plant in the order $s > g > a$ and $MTT > BOG > PSB > LAU > NCE$.

Multiple-choice experiment

The five populations showed no significant difference in their distribution of eggs across the four plant species (Fig. 3, $F_{12,201} = 1.01, P = 0.44$) and no difference in the strength of preference for any one species (for a, $F_{4,66} = 1.82, P = 0.14$; for g, $F_{4,66} = 0.77, P = 0.55$; for s, $F_{4,66} = 0.28, P = 0.89$). They did, however, show strong preferences among the three ‘normal’ host species ($F_{2,134} = 49.98, P < 0.001$), with a clear preference for *C. spinosissimum* in all populations.

Considering just the binary decision to lay eggs on a plant or on the box, there were highly significant differences between populations (Fig. 3, $F_{4,67} = 6.57, P < 0.001$). They fell into two groups: BOG, MTT and PSB, which laid an average of 76% of their eggs on the plant; and LAU and NCE, which laid an average of 44%. These groups differed significantly ($F_{1,67} = 23.0, P < 0.001$), but within a group there was no significant variation ($F_{3,67} = 1.10, P = 0.36$).

Discussion

There was little geographic variation in oviposition preference, and hence no evidence of local adaptation to differences in host availability despite isolation of populations and severe time limits

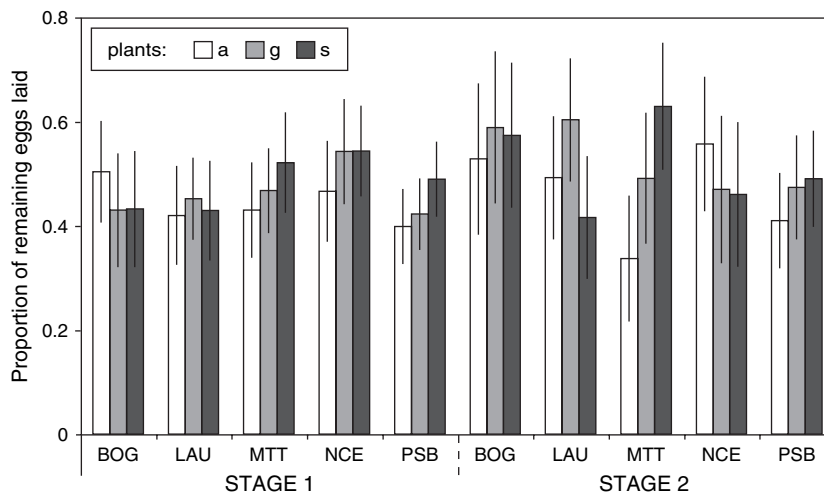


Fig. 1. The proportion n/m of her remaining eggs laid by each female during the first two stages of the no-choice experiment. Mean proportions and standard errors were obtained by back-transformation of R output. Plants presented: *A. alliariae* (a), *A. glabra* (g), *C. spinosissimum* (s). Five populations coded as in Table 1.

Table 2. Analysis of variance on the results from the no-choice experiment. The rows labelled null give the original variances, with lower rows giving the reductions that correspond to the inclusion of differences between stages (Stage), beetle populations (Pop), plant species (Plant), and the interaction of population and plant type (Pop \times plant). The final column shows the significance of the reductions due to these effects.

	d.f.	Reduction in variance	<i>F</i>	<i>P</i>
Proportion of remaining eggs laid during each stage				
Null	536	1671.04		
Stage	1	7.76	2.94	0.087
Pop	4	20.62	1.96	0.100
Plant	2	15.95	3.03	0.049
Pop \times plant	8	31.62	1.50	0.155
Proportion of eggs laid on the plant				
Null	745	3571.4		
Stage	2	9.8	1.54	0.215
Pop	4	152.7	11.95	< 0.001
Plant	2	568.6	88.97	< 0.001
Pop \times plant	8	47.3	1.85	0.065

on the breeding season that should promote it in this system. All five *O. elongata* populations showed a very strong preference for *C. spinosissimum* when given a simultaneous choice between hosts. Furthermore, contact with either *A. alliariae* or *A. glabra* in nature did not make a population more likely to rank that species in second place. Contrary to predictions, specificity also showed very little divergence between populations: in the no-choice experiment, females from all populations laid $\approx 47\%$ of their remaining eggs during each stage, almost regardless of the host species presented. Only in the curious behaviour of laying eggs on the box were there strong differences, with a greater proportion laid there by beetles from LAU and NCE in both experiments, and by all populations in the no-choice experiment when presented with *Adenostyles*, particularly *A. alliariae*.

In other species, tests for geographic variation in oviposition preference have given mixed results. Changes in specificity are more common than alterations to plant ranking (Jaenike, 1990; Funk & Bernays, 2001). Both can evolve rapidly (Thomas *et al.*, 1987; Singer *et al.*, 1993) and can differ even between sympat-

ric populations (Prokopy *et al.*, 1988; Craig *et al.*, 1993; Via, 1999; Linn *et al.*, 2003), yet species sometimes show conserved preferences (Thompson, 1993; Wehling & Thompson, 1997). Several explanations have been proposed for conservatism (Thompson, 1993; Wehling & Thompson, 1997). Firstly, it may just result from a lack of time to adapt, as in the responses of several butterfly species to introduced plants (Tabashnik, 1983; Bowers *et al.*, 1992). Secondly, high rates of gene flow may constrain divergence (Jaenike, 1989), because the extent of local adaptation depends on the balance between gene flow and selection (Barton & Whitlock, 1997; Lenormand, 2002). Thirdly, genetic correlations may limit the extent to which preferences for different plants can evolve independently (Thompson, 1993), and finally, preference may simply become a neutral trait in the absence of the preferred host if females are sufficiently motivated to accept other suitable plants (Jaenike, 1990).

For *O. elongata*, the latter explanation seems to apply. The no-choice experiment suggests that there is only a very shallow gradient of preference among the three plant species. The level of

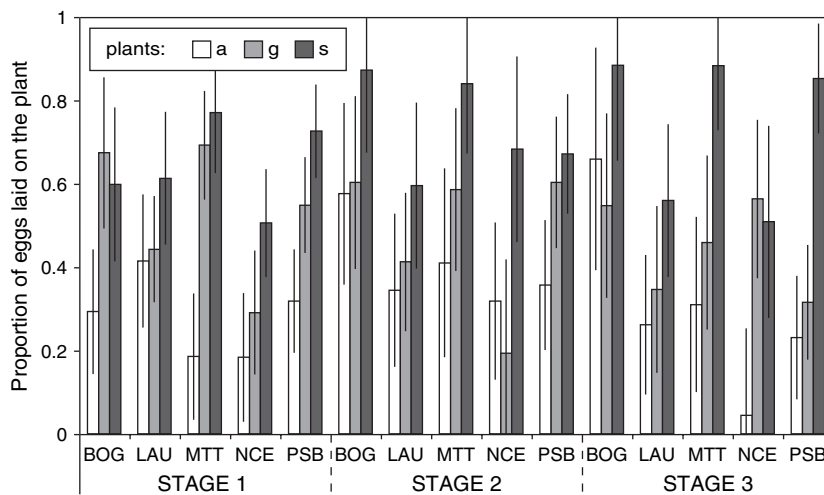


Fig. 2. The proportion of eggs laid on the plant (rather than on the box) during each stage of the no-choice experiment. Mean proportions and standard errors were obtained by back-transformation of R output. Coding as in Fig. 1 and Table 1.

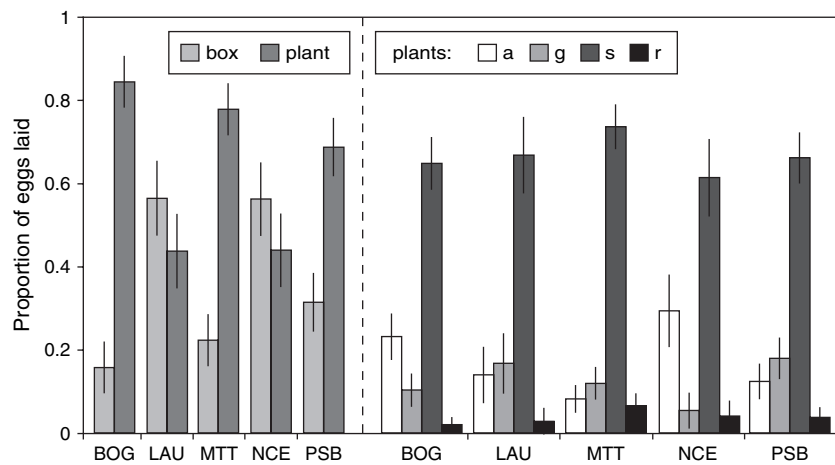


Fig. 3. Results from the multiple-choice experiment, showing the means and standard errors for the proportion of eggs laid on the box versus on a plant, and the proportions among the four plants with the box excluded. Populations and plants coded as in earlier figures, with the addition of *Rumex alpinus* (*r*).

specificity is strong enough that most eggs are laid on *Cirsium* in simultaneous choice experiments and in the wild (Ballabeni *et al.*, 2001a). Yet in the absence of a choice, females of all populations are sufficiently motivated to oviposit on any of the three host plants rather than waiting for *Cirsium*. This would remove the tendency for host ranking or specificity to diverge in populations that lack this host, as the selection pressure induced by wasting time is absent. The fine gradient of preference may have evolved as a general response to the patch fidelity and typical habitat of *Oreina* species, where ovipositing females find themselves in patches dominated by suitable host plants (Ballabeni *et al.*, 2001a). The ecology of the beetle may therefore circumvent the selection pressure for local adaptation, by making preferences among the three suitable hosts effectively neutral in most populations.

The other explanations for conservatism are less likely to hold.

1. There appears to have been sufficient time for local adaptation, as the populations have been separated long enough to generate a high F_{ST} and to diverge in larval growth rates and foraging behaviour (Ballabeni *et al.*, 2003; Gotthard *et al.*, 2005).
2. Gene flow is clearly not preventing divergence. The populations span a distance of about 270 km and are very strongly isolated, as, although the beetles live at high altitude, they are still separated by the soaring peaks and plunging valleys of the Alps. This results in genetic isolation, with microsatellite allele frequencies giving high pairwise F_{ST} everywhere and an overall value of 0.379 (Margraf, 2003), implying around two effective migrants between populations every five generations ($Nm = 0.410$). In fact, the very high value of F_{ST} probably represents a complete absence of migration and the gradual decay of genetic similarity since population separation (Whitlock & McCauley, 1999), rather than constant very low migration, as this species has never been known to fly. Populations of *O. elongata* are therefore free to adapt independently to local conditions, unhindered by gene flow.
3. Finally, nothing is known about genetic correlations between the preference for different plants (the life-cycle

of *O. elongata* makes this difficult to study, because they pass a second season as a non-reproductive adult before breeding). However, in other species the levels of acceptance for different host plants are often negatively correlated (e.g. Hawthorne & Via, 2001; Dambroski *et al.*, 2005), which would actually promote divergence in isolated one-host populations.

The finding of no divergence in specificity differs from the conclusion drawn in a previous study which used beetles from LAU, MTT and PSB (Gotthard *et al.*, 2004). Those results were interpreted as showing local adaptation of lower specificity in the population from LAU, which in the wild does not encounter the preferred host, *Cirsium*, and so must accept other plants. Their analysis differs in that the proportions of eggs laid on different hosts in no-choice experiments were calculated after exclusion of those laid on the box. However, it seems reasonable to assume that, in this confined space, all oviposition was motivated by the particular plant species present (an argument supported by the significant effect of plant in Table 2). Inclusion of the eggs laid on the box in their calculations gives a result identical with that found here: populations do not differ. In both sets of experiments, therefore, specificity has not evolved in response to differences in the host plants available.

A preference for *Cirsium* was expected in populations that encounter it naturally. It is seen in the wild at the Col du Petit Saint-Bernard site, where more eggs are laid on *Cirsium* than on *A. alliariae* (Ballabeni *et al.*, 2001a). This seems to be a response to the lower level of egg predation on *Cirsium*, which results from the protection offered by the spines and complex shape of its leaves (Ballabeni *et al.*, 2001b). In contrast, differences in larval performance on different hosts will have little influence on oviposition preference. There is some evidence for minor local adaptation of growth rates (Ballabeni *et al.*, 2003), but because the larvae are highly mobile and move repeatedly between plant species when two are present (Gotthard *et al.*, 2005), there is no opportunity for larval performance to impose selection on oviposition choice. The preference-performance correlation is therefore related to enemy-free space during the egg stage rather than to nutritional value. The fact

that all populations showed the same very strong preference is contrary to any prediction from local adaptation. It suggests that beetles from LAU and NCE developed this preference while in contact with *Cirsium* in the past, either through founding of these populations from sources with *Cirsium*, or *in situ* before its loss from both sites.

Two other results may also be related to the higher survival of eggs on *C. spinosissimum*. In no-choice trials, all populations laid more eggs on the box when *Cirsium* was absent, and, in both types of experiment, beetles from LAU and NCE laid a greater proportion of their eggs on the box than other populations. Eggs can be found off the host plant in the wild, and in field trials in which females were placed in nylon cages on plants but with access to the ground, 26% of their eggs were laid on the ground and 35% on the cage (unpublished results). Laying away from the plant may be an adaptation to avoid egg loss to predators that are attracted to the plant, with the behaviour being most common in LAU and NCE, the two populations where the safest host, *Cirsium*, is not available. This argument can perhaps be taken further, to explain a shift in reproductive mode in the group of species most closely related to *O. elongata*, in which oviparity seems to be ancestral (Dobler *et al.*, 1996). Both *Oreina cacaliae* and *Oreina speciosissima* are viviparous and they never (*O. cacaliae*) or rarely (*O. speciosissima*) feed on *C. spinosissimum*. It is unclear why this reproductive mode has evolved in these two species, as the larvae are produced at a similar size to those of *O. elongata* because, unlike another viviparous clade within *Oreina*, they receive no extra nutrition from the mother (Dobler *et al.*, 1996). Viviparity and off-plant oviposition may in fact represent alternative strategies to avoid egg predation when using host plants other than the mechanically protected *C. spinosissimum*.

Oreina elongata does not show the expected local adaptation of host ranking and specificity in response to differences in host availability in these scattered, highly isolated alpine populations. Instead, the species as a whole retains an oviposition preference for a plant that offers enemy-free space during the egg stage. This is combined with a shallow gradient of preference across other hosts, resulting in their rapid acceptance in the absence of the safest species, an essential feature under the time constraints of the brief alpine summer. This conservatism means that populations largely remain ecologically equivalent, but not because of recent recolonisation. Looking more widely within the genus, tritrophic interactions between the beetles, their host plants, and their predators, in addition to their more obvious role in chemical defence, seem capable of driving the evolution of oviposition behaviour and reproductive mode.

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