The effect of plant population size on the interactions between the rare plant *Gentiana cruciata* and its specialized herbivore *Maculinea rebeli*

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Summary

1 Many rare plant species are restricted to small isolated populations in which fitness may be reduced because of inbreeding, environmental and demographic stochasticity, and reduced pollination. However, specialist herbivores are less likely to be present in such populations because of higher probabilities of herbivore extinction and lower rates of colonization, and may therefore affect fitness only in larger plant populations.

2 We studied the relationships between the size of populations of the endangered grassland plant *Gentiana cruciata* and the probability of occurrence and population size of its specialist herbivore, the endangered butterfly *Maculinea rebeli*, and their effects on plant size, fruit herbivory and seed production.

3 The 29 *G. cruciata* populations studied ranged in size from 1 to 337 genets and 18 of them supported a *M. rebeli* population. *M. rebeli* populations were both more likely and larger in larger *G. cruciata* populations. Estimated adult herbivore populations were small, ranging from 1 to 42 individuals, with a median of 11. We conclude that the conservation of *M. rebeli* requires the conservation of large *G. cruciata* populations.

4 Although large populations of *G. cruciata* produced more flowers, a greater proportion of their fruits were attacked by herbivores. Fruit herbivory, which considerably decreased the number of seeds per fruit, appears to have been caused largely by *Maculinea*. The number of seeds both per fruit and per genet significantly decreased with the number of *M. rebeli* eggs per genet. The overall independence of *G. cruciata* seed production from population size may result from the opposing effects on fruit production and herbivory.

5 Our study suggests that complex interactions between different trophic levels may determine the population dynamics of rare species. Furthermore, small population size may have both negative and positive effects on the fitness of endangered species.

Key-words: biotic interactions, conservation biology, habitat fragmentation, herbivory, population size, reproduction


Introduction

In Central Europe, semi-natural habitats such as extensively managed calcareous grasslands have become increasingly fragmented as a result of changes in human land use (Zoller & Wagner 1986; Keymer & Leach 1987). Plant and animal species that depend on this type of habitat have therefore become restricted to populations that are smaller in size and area occupied, and which are more isolated, than in the past and may risk reduced survival and reproduction. Environmental and demographic stochasticity may reduce the probability of persistence of small populations and increased inbreeding may reduce plant growth, survival and reproduction compared with large populations (Oostermeijer et al. 1994; Fischer & Matthies 1998a,b; Kéry et al. 2000), while pollination probability may also be reduced (Byers 1995; Ågren 1996).

Although isolation and small population size may endanger persistence, they may also have beneficial effects on fitness; for instance, if specialist herbivores
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and pathogens are less likely to survive in or locate such populations (Grevstad 1996; Zabel & Tscharntke 1998; Ericsson et al. 1999). However, the conservation of specialist herbivores which depend on rare and declining plant hosts then becomes a problem.

We studied the effects of population size on the biotic interactions between Gentiana cruciata, a plant of extensively managed calcareous grasslands, and the butterfly Maculinea rebeli, for which it is the exclusive food host. Both species are endangered in many parts of Central Europe and occur only in highly isolated and often very small populations (Gonseth 1987; Landolt 1991; Hochberg et al. 1992; Korneck et al. 1996).

Maculinea rebeli has a parasitic life cycle. The caterpillars spend the first weeks of their life on the host plant, feeding from flowers and fruits, before dropping to the ground. They are then carried into the nest of their ant host species, where they grow, pupate and emerge. While the butterfly–ant interactions have been comprehensively studied (e.g. Hochberg et al. 1994; Elmes et al. 1996; Thomas et al. 1998; Van Dyck et al. 2000), little is known about the butterfly–plant interactions (Elmes et al. 1996).

We studied 29 populations of G. cruciata in the Swiss and French Jura mountains to determine whether (i) there is a positive relationship between the size of G. cruciata populations and the probability of occurrence, and population size, of M. rebeli, (ii) fruit herbivory adversely affects the reproduction of G. cruciata, (iii) there is a positive relationship between the size of G. cruciata populations and the incidence of fruit herbivores in general, and (iv) there is a positive relationship between the size of G. cruciata populations and reproduction.

Methods

STUDY SPECIES

Gentiana cruciata L. (Gentianaceae), the cross-leaved gentian, has a Euro-West-Siberian, submediterranean/montane distribution (Hegi 1926; Meusel et al. 1978) and is typical of nutrient-poor calcareous grasslands (Zoller & Wagner 1986). In July, this perennial herb typically produces 1–4 stems, each with 10–20 dark-blue flowers, that are 20–30 cm tall. On average, fruits contain about 100 seeds (personal observation by M. Kéry). Gentiana cruciata is self-compatible but produces very few seeds in the absence of pollination by bumblebees, hoverflies and butterflies (Petanidou et al. 1995). The flight period of M. rebeli only partly overlaps G. cruciata flowering and it cannot therefore be an important pollinator and is indeed seldom seen on the open flowers (J.A. Thomas, personal communication).

The large blue butterfly Maculinea rebeli (Hirschke) has a Euro-West-Siberian, submediterranean/montane distribution (Gonseth 1987), is unvoltine, and has been reported as living in closed populations (Hochberg et al. 1996). The habitat specificity of its host plant restricts M. rebeli to calcareous grassland where it is considered endangered both in Europe as a whole (Wells et al. 1983) and in Switzerland (Gonseth 1987).

In June, females lay about 150 conspicuous white eggs on the developing inflorescences of host plants (Hochberg et al. 1992). Although Gentianella germanica has been suggested as an alternative host plant by some authors (Gonseth 1987; Lepidopteren-Arbeitsgruppe 1987) – but not by others (e.g. Hochberg et al. 1994; Thomas et al. 1998) – and despite the fact that it co-occurred with G. cruciata at 25 of our 29 sites, we never found any M. rebeli eggs on it.

The first three larval instars of M. rebeli feed in the flowers and on developing fruits of G. cruciata. There is rarely more than one caterpillar per flower (Elmes et al. 1996). After 3 weeks, caterpillars moult for the last time and drop off the inflorescence. They may then be found by ants of the species Myrmica schencki, which are deceived by pheromones into adopting the caterpillars, taking them into their nest. Here they are fed on regurgitations, trophic eggs and prey, before emerging in the following May–June. Maculinea rebeli may therefore harm the reproduction of both its food plant G. cruciata and its ant host M. schencki. Colonies of M. schencki are only parasitized if they are within 2 m of the nearest G. cruciata plant. If densities of G. cruciata are higher than 1000–1500 genets ha−1, few ant colonies escape parasitism that the population density of M. rebeli is decreased (Hochberg et al. 1994; Elmes et al. 1996).

STUDY SITES

We studied a total of 29 G. cruciata populations in July–August of either 1998 or 1999 (Table 1). Two of the 1998 populations (Coulo Berg and Kleinlützel B) consisted of a single genet and could not be relocated in 1999. They were replaced by two new populations in 1999 (Les Mayettes and Ste-Colombe).

Most populations were situated in the Jura mountains south and west of the city of Basel, Switzerland (47°30’ N, 7°35’ E) and two near the town of Pontarlier (46°50’ N, 6°20’ E). The two easternmost populations (Gwiden and Bläwidel) were outside the current range of M. rebeli (Gonseth 1987). The populations grew in nutrient-poor calcareous grassland, either in extensively grazed pastures or in mown meadows. The distance from one study population to its nearest known neighbour population ranged from 0.13 km to 22.4 km with a median of 1.8 km.

SITE CHARACTERISTICS, SIZE AND AREA OF POPULATIONS OF G. CRUCIATA

We read longitude, latitude and altitude of the study sites from 1:25 000 maps. In 1998 (and in 1999 for the two populations located only in 1999), we noted the type of land use at each site. We assessed the size of each population of G. cruciata by counting the number of flowering genets. To estimate the area occupied by a population, we marked the position of the outermost
genets on a map, and measured the area of the convex polygon defined by these genets. This alternative measure of population size was highly correlated with the number of flowering genets ($r^2 = 0.74$, $P < 0.001$).

In 1998 and 1999 we selected, where possible, a random sample of up to 40–100 genets in each population of *G. cruciata* by locating each genet within 1 m of a transect across the maximal extension of the population. In populations consisting of less than 20 genets, we sampled all genets. For each selected genet, we counted the number of flower stems. We measured the height and counted the number of flowers on the first recorded stem of each genet and, for a subsample of 20–40 genets (all genets in small populations), scored it for the presence of *M. rebeli* eggs. If there was at least one of the unmistakable white eggs present on any of the genets, we considered the *G. cruciata* population to be associated with a *M. rebeli* population.

The size of the adult population of *M. rebeli* was estimated by counting the number of eggs on the stems sampled in 1999 and multiplying the mean number of eggs per stem by the mean number of flower stems per genet and the mean number of genets per population. We assumed an even sex ratio and a clutch size of 150 eggs per *M. rebeli* female (Hochberg et al. 1992) and therefore divided this value by 75 to obtain the number of adult butterflies.

### Table 1

The 29 study sites with populations of *Gentiana cruciata* in the Swiss and French Jura Mountains. Longitude and latitude are based on the coordinate system of the Swiss Federal Office for Topography (Wabern, Switzerland). Sites with a zero entry in the last column did not support a population of *Maculinea rebeli*. The last two populations are outside the current distributional range of *M. rebeli* (Gonseth 1987).

<table>
<thead>
<tr>
<th>Site</th>
<th>Longitude (km)</th>
<th>Latitude (km)</th>
<th>Altitude (m a.s.l.)</th>
<th>Habitat</th>
<th>Genet number of <em>G. cruciata</em></th>
<th>Proportion of genets with <em>M. rebeli</em> eggs</th>
<th>Number of eggs of <em>M. rebeli</em> (1999 only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Les Mayettes</td>
<td>502.400</td>
<td>192.400</td>
<td>840</td>
<td>Pasture</td>
<td>2800</td>
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<tr>
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<td>193.200</td>
<td>780</td>
<td>Pasture</td>
<td>7500</td>
<td>0.49</td>
<td>6506</td>
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<td>249.850</td>
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<td>Meadow</td>
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<td>0.74</td>
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<td>236.600</td>
<td>980</td>
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<td>760</td>
<td>Pasture</td>
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<td>1.00</td>
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<td>0</td>
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<td>Gwidem</td>
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<td>Pasture</td>
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<td>337</td>
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<td>530</td>
<td>Meadow</td>
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</table>
Interaction between a rare gentian and a butterfly

DATA ANALYSIS
Most of our data on plant size and reproduction of G. cruciata, and on the presence and the number of eggs of M. rebeli on genets or fruits of G. cruciata had an unbalanced and hierarchical structure, with fruits nested within genets and genets nested within populations. We consider fruits, genets and populations as random factors and therefore applied a mixed modelling approach, using the restricted maximum-likelihood (REML) method (Patterson & Thompson 1971). Effects of fixed factors (such as G. cruciata population size, presence of a M. rebeli population, number of M. rebeli eggs, stem length, year, herbivore presence in a fruit, and their interactions) were tested with the Wald test (Dobson 1990). Effects of random factors (variance components) were tested with a likelihood ratio test (Morrell 1998), which compares the deviances of a model with and without the component. The difference in deviance is approximately χ²-distributed on 1 d.f. (Littell et al. 1996). Frequency data such as the presence of M. rebeli eggs on genets within populations and the proportion of fruits attacked by herbivores were analysed using a generalized linear mixed model with logit link and binomial errors (Sealre et al. 1992; Breslow & Clayton 1993), and with genets nested in populations as random effects.

Data on the occurrence of M. rebeli in a population of G. cruciata were analysed with a logistic regression model. We tested the statistical significance of factors by assuming mean deviance ratios as approximately F-distributed (McCullagh & Nelder 1989). To obtain a 95% confidence interval for the critical gentian population size necessary to support a population of G. cruciata, we report the median and the quartile range (qr; 25th–75th percentile). All analyses were carried out with the statistical software package Genstat (Version 5.4.1., Payne et al. 1993).

Results
SITE CHARACTERISTICS AND POPULATION SIZE OF G. CRUCIATA
Nineteen out of the 29 study sites were grazed by cattle and the remaining 10 were mown (Table 1). Altitude ranged from 420 to 1000 m a.s.l., with pastures significantly higher than meadows (767 vs. 629 m a.s.l.; t₁₅ = 2.03, P = 0.052). The distribution of G. cruciata population sizes was lognormal ranging from 1 to 337 genets. Most populations were very small, with median values for size and area occupied of only 12 genets (qr: 4–41) and 0.2 ha (qr: 0.04–1.0). Median population density was 95 genets ha⁻¹ (qr: 30–180). Population size was independent of land-use type (t₁₅ = −1.13, P = 0.292) and altitude (t₁₅ = 1.08, P = 0.292).

OCURRENCE AND SIZE OF M. REBELI POPULATIONS
Overall, we recorded M. rebeli populations in 18 out of the 27 (66.7%) G. cruciata populations within its range (14 of the 25 studied in 1998, 17 out of the 25 in 1999). There was a change from presence to absence of M. rebeli or vice versa, between years at three sites. One G. cruciata population, which had 23 genets and was situated 2.6 km from the nearest known population, supported M. rebeli in 1999 but not in 1998. Two neighbouring G. cruciata populations, with 6 and 8 genets, respectively, that were situated 300 m from each other, switched M. rebeli occupancy status from 1998 to 1999; one gained and the other lost a population. The probability that M. rebeli occurred at a site increased with the population size of its host plant (approximate F₃₅ = 26.86, P < 0.001; Fig. 1a), with a critical population size for G. cruciata to support a M. rebeli population with 95% probability of 21 genets (C.I. = 12–70). However, eggs were present in populations with as few as 2 genets, and 28% of M. rebeli populations occurred on populations consisting of 10 or fewer genets of G. cruciata. The probability of M. rebeli occurrence was independent of altitude (approximate F₃₅ = 0.48, P = 0.37), but was higher in pastures than in meadows (77.8 vs. 33.3%; approximate F₃₅ = 4.15, P = 0.052). The effect of G. cruciata population size remained significant after eliminating the effect of habitat type (approximate F₃₅ = 26.86, P < 0.001).

In those G. cruciata populations that supported M. rebeli, a mean of 73% of genets had eggs present, independent of population size (Table 2a, Fig. 1b). The probability that M. rebeli eggs were present increased with the length of a flower stem (Table 2a). We found a median of 8.5 M. rebeli eggs (qr: 0–19) on each flower stem, corresponding to a median of 0.375 eggs per fruit (qr: 0–0.833) and 18 eggs per genet (qr: 0–57). Significantly more eggs were laid on longer flower stems (Table 2b) and marginally significantly more on G. cruciata genets in larger populations (Table 2c).

We estimated a median of 804 eggs per M. rebeli population (qr: 150–3155), which corresponds to a median estimated adult population size of M. rebeli of only 11 butterflies (range: 1–190, qr: 2–42). Larger G. cruciata populations supported larger M. rebeli populations (Fig. 1c; polynomial regression: linear, F₁₅ = 29.33, P < 0.001; quadratic, F₁₅ = 9.07, P = 0.006).
Genets had a median of 2 flower stems (qr: 1–4) with an average length of 24.6 cm (SD = 8.0) and a median number of 15 flowers (qr: 9–24) per stem, which corresponds to 30 fruits per genet (qr: 14–72). Genets produced significantly fewer, but taller stems, and more flowers both per individual stem and per genet in 1999 than in 1998. Genets in larger populations produced more flowers per genet ($P = 0.058$, Table 3, Fig. 2). This effect was more marked in 1999 than in 1998 (see significant year-by-population size interaction in Table 3d).

Signs of fruit herbivory were present in a median of 20.0% of all fruits (qr: 0–50.0%) and the proportion increased with $G. cruciata$ population size (Table 4, Fig. 1).
Fig. 1d). The mean proportion of damaged fruits was much higher in populations with *M. rebeli* present than in populations without (33.0 vs. 3.8%; \( t = 4.46, P < 0.001 \); see also Table 4) and was similar to the proportion estimated of the 0.375 *M. rebeli* eggs laid per fruit (see above) and assuming they suffered no mortality. This suggests that the major part of the fruit damage detected had been caused by *M. rebeli*, although there

Table 3 The effect of plant population size and different years on genet size and reproduction in 29 populations of *G. cruciata*. The analyses are based on mixed model analysis with REML estimation. All variables except stem height were log-transformed. Sample sizes were as follows: (a) 1292, (b) 818, (c) 815, and (d) 814 genets

<table>
<thead>
<tr>
<th>Source</th>
<th>(a) No. flower stems per genet</th>
<th>(b) Stem length</th>
<th>(c) No. flowers per stem</th>
<th>(d) No. flowers per genet</th>
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<td></td>
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<td>( p )</td>
<td>Effect</td>
</tr>
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<td>Population size</td>
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<td>2.0</td>
<td>0.157</td>
<td>-0.383</td>
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<tr>
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<td>15.520</td>
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<tr>
<td>Year (1999 vs. 1998)</td>
<td>-0.031</td>
<td>5.4</td>
<td>0.020</td>
<td>1.708</td>
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</tbody>
</table>
| Year \(
\times \) population size | 0.054 | 2.6 | 0.107 | 1.001 | 1.3 | 0.254 | 0.035 | 1.2 | 0.273 | 0.113 | 3.9 | 0.048 |
| Residual genet variance component | 0.069 | 42.050 | 0.066 | 0.178 |

Table 4 The effect of population and genet size of *G. cruciata*, and the presence of *M. rebeli*, on the proportion of fruits attacked by herbivores. Analysis based on a generalized linear mixed model analysis with REML estimation (\( n = 172 \) genets in 26 populations). Fixed effects were tested using the Wald test (Dobson 1990) and random effects with a likelihood-ratio test (see Methods for details).

<table>
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<tr>
<th>Source</th>
<th>Effect</th>
<th>( \chi^2 )</th>
<th>( p )</th>
</tr>
</thead>
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<td>6.5</td>
<td>0.011</td>
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<tr>
<td>Population size of <em>G. cruciata</em> ( \times ) presence of <em>M. rebeli</em> population</td>
<td>-0.746</td>
<td>0.0</td>
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</tr>
<tr>
<td>Stem length of <em>G. cruciata</em> ( \times ) presence of <em>M. rebeli</em> population</td>
<td>0.113</td>
<td>0.1</td>
<td>0.752</td>
</tr>
<tr>
<td>Residual genet variance component</td>
<td>-0.115</td>
<td>3.1</td>
<td>0.078</td>
</tr>
</tbody>
</table>

**Fig. 2** The relationship between population size of *G. cruciata* and (a) the mean number of flower stems per genet, (b) the mean length of flower stems, (c) the mean number of flowers per flower stem, and (d) the mean number of flowers per genet. For significance tests, see Table 3.
was no direct way to distinguish such herbivory from that due to other insects because the caterpillars had left the plants by the time we collected fruits. 

G. cruciata genets developed on average 87.1 (SD = 42.9) seeds per fruit regardless of population size (Table 5a). Fruits contained on average 70 seeds at sites supporting a population, as compared to 123 developed seeds per fruit at sites without the butterfly (P = 0.114). Each M. rebeli egg reduced seed number per fruit by 8.45 (Table 5a). The number of developed seeds per fruit increased with stem length, indicating either greater pollination rates or more resources available per fruit to larger plants, but the slope of this relationship was significantly smaller at sites with M. rebeli (interaction in Table 5a). There was a marked effect of herbivores on seed development. Fruits that showed signs of herbivore presence produced only 68 seeds, compared to 123 developed seeds in intact fruits (P < 0.001, Table 5a). This reduction was more marked at sites that supported a M. rebeli population, further suggesting that M. rebeli is the most important fruit herbivore of G. cruciata.

The median estimated number of developed seeds per genet, 2,366 (qr: 926–6,534), was independent of population size (Table 5b). The number of developed seeds per genet was greater where plants were larger (positive effect of stem height) but not more developed seeds per fruit or genet. Herbivore presence considerably reduced the number of seeds developed per fruit, with numbers both per fruit and per genet negatively correlated with numbers of M. rebeli eggs. The proportion of fruits damaged by herbivores was higher in larger populations of G. cruciata.

### Discussion

#### Occurrence and Size of Populations of M. rebeli

Populations of the endangered herbivorous butterfly M. rebeli were both more likely to occur and to be larger in size in larger populations of its host plant G. cruciata. This suggests that the probability of M. rebeli becoming extinct at a particular site or of it colonizing would depend on the host population size. Generally, small insect populations are at a higher risk of extinction due to chance events than large populations (Thomas & Jones 1993; Greystad 1996). The small populations of G. cruciata studied probably constitute relics of once larger populations and those which are currently without M. rebeli may well have lost an associated butterfly population during their decline.

Once a small G. cruciata population has lost its M. rebeli population, it is probably less likely to be recolonized than a large population. Area occupied was highly correlated with the number of plants, and smaller G. cruciata populations thus presented a smaller target for dispersing individuals of M. rebeli. Similar positive relationships between host population size and the occurrence of associated species have been reported.
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for other herbivores (e.g. a moth: Forare & Solbreg 1997; 11 species of monophagous plant bugs, leaf hoppers, and butterflies: Zahal & Tischstein 1998), as well as for parasitoid wasps (Lei & Hanski 1998; Van Nouhuys & Hanski 1999) and for pathogens (the anther-smut disease: Carlsson & Elmqvist 1992; Carlsson-Granér et al. 1998; two rust pathogens: Burdon et al. 1995; Ericson et al. 1999).

We found a close positive relationship between the population size of G. cruciata and that of M. rebeli, in line with empirical data from butterfly populations in France and Spain (Elmes et al. 1996) and with predictions based on a model of the interactions between G. cruciata, M. rebeli and the ant M. schencki, among 19 variables, abundance of G. cruciata had the greatest power to predict abundance of M. rebeli (Hochberg et al. 1994).

Two sites were newly colonized by M. rebeli in the second study year, one of them over a distance of at least 2.6 km, the other over a distance of at least 300 m. This is inconsistent with reports that M. rebeli lives in closed populations (e.g. Hochberg et al. 1996). However, two butterfly species which are considered to have a metapopulation structure, Lyciaedus melissa (Knutson et al. 1999) and Hesperia comma (Hill et al. 1996), both had a maximum recorded dispersal distance of c. 1 km and such a structure could explain our results with M. rebeli.

The median adult population size of M. rebeli here (11) was very small. However, most of 14 populations in France and Spain studied by Elmes et al. (1996) (calculated from their Table 2) were clearly larger and ranged in size from 6 to 2539 individuals, with a median size of 45. If our estimates of adult population size are correct, many of the M. rebeli populations may be threatened by extinction in the near future, even if the very low annual variations in abundance of this species (Thomas et al. 1998) may allow survival in remarkably small populations.

Relationship between plant size and population size in G. CRUCIATA

Genets of G. cruciata in large populations produced more flowers than genets in small populations, although plant size did not differ. Insofar as plant size may be interpreted as an integrative measure of habitat quality for established plants, this suggests that reduced population size was not the result of poorer growing conditions as was suggested by Widén (1993).

Habitat quality cannot therefore explain the positive relationship between the number of flowers per plant and population size; an alternative cause may be genetic erosion in small populations (Young et al. 1996). Although to date there have been few experimental studies on the relationship between measures of fitness and population size in plants, positive correlations were attributed to genetic erosion in Gentiana pneumonanthe (Oostermeijer et al. 1994), Gentianella germanica (Fischer & Matthies 1998a,b) and Primula veris (Kéry et al. 2000).

Herbivory and reproduction in G. CRUCIATA, AND THEIR RELATIONSHIP TO THE SIZE OF PLANTS AND OF PLANT POPULATIONS

Herbivory reduced seed number per fruit in G. cruciata by more than 50% and modified the positive relationship between plant size and reproduction (reflected in the significant interaction between stem length and the presence of M. rebeli in Table 5a). Thus, large plants produced more seeds than small ones, but the difference was less when M. rebeli was present. The butterfly lays eggs preferentially on genets with long stems (Table 2) and there may therefore be selection against large G. cruciata plants in populations which support M. rebeli.

Genets of G. cruciata in large populations suffered more from fruit herbivory, which considerably reduced the number of developed seeds per fruit. The much higher levels of herbivory in populations with M. rebeli and the absence of other specialist fruit herbivores known to depend on G. cruciata, suggest that this species, rather than another co-occurring herbivore is the major cause of damage.

The probability that a G. cruciata population was infested with M. rebeli increased with increasing plant population size. Genets in larger populations, however, had more flowers and this may compensate for the negative effects of herbivory leading to the number of developed seeds per genet being independent of plant population size.

Conclusions

Maculinea rebeli was more likely to occur, and the number of butterflies was higher, in larger populations of Gentiana cruciata, indicating that the successful conservation of the endangered herbivore requires large populations of its host. Large population size may also be important for the persistence of G. cruciata itself because it serves as a buffer against environmental stochasticity (Fischer & Stöcklin 1997). Moreover, if the reduced number of flowers per genet of G. cruciata in small populations is an effect of population size itself, large population size would also increase individual plant fitness. Conservation efforts aimed at increasing the area of suitable habitat for G. cruciata might also increase the likelihood of the occurrence of M. rebeli, because at least one unoccupied site was colonized from a distance of several kilometres.

Our study illustrates that understanding biotic interactions is important for understanding the ecology of plant and animal species (Waser & Price 1998). The local survival of one rare species should not be viewed in isolation because it may also determine the fate of other associated rare species.
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