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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

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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; Ericson *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-Westsiberian, submeridional/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-Westsiberian, submeridional/montane distribution (Gonseth 1987), is univoltine, 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}, so 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 (Gwidem and Bälweid) 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

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)

Site	Longitude (km)	Latitude (km)	Altitude (m a.s.l.)	Habitat	Area (m^2)	Genet number of *G. cruciata*	Proportion of genets with *M. rebeli* eggs	Number of of eggs of *M. rebeli* (1999 only)
Les Mayettes	502.400	192.400	840	Pasture	2800	161	0.87	14266
Ste-Colombe	511.400	193.200	820	Pasture	75000	303	0.49	6506
Chevenez	568.875	249.850	460	Meadow	8000	70	0.74	1878
Ecos	587.500	236.600	980	Meadow	11000	140	0.58	1286
Coulu	590.750	236.100	760	Pasture	20000	50	1.00	6621
Mowe	590.750	251.450	780	Pasture	400	2	0	0
Coulo Berg	591.075	236.450	930	Pasture	100	1	0	0
Coulu rechts	591.100	236.150	770	Pasture	1000	10	1.00	650
Coulo Weg	591.150	236.350	860	Meadow	250	4	0	0
Moweo	591.200	251.550	830	Pasture	400	8	0.38	46
Hornis	591.350	255.650	760	Pasture	10000	30	1.00	1601
Moha	591.500	251.500	770	Meadow	6000	6	0	0
Kica Wiese	592.300	254.550	520	Meadow	2000	19	0.82	402
Hasenschell	594.650	251.250	710	Pasture	10000	192	0.96	9315
Soyhières L	594.700	250.900	530	Pasture	2000	36	1.00	3155
Soyhières R	595.750	250.950	540	Pasture	27000	41	0.45	133
Kleinlützel B	597.500	253.200	550	Pasture	100	1	0	0
Liesberg	598.400	250.400	520	Pasture	100	23	0.15	49
Vicques	599.200	246.150	630	Pasture	12000	12	0	0
Kleinlützel O	599.275	252.900	430	Meadow	400	6	0	0
Leymen	603.200	259.850	420	Meadow	100	2	0	0
Les Pouches	608.000	243.375	970	Pasture	20000	12	0.75	804
Erschwil	608.525	246.850	840	Meadow	650	6	0	0
La Neuve Vie	608.800	243.800	950	Pasture	20000	20	0.69	380
Trottelreben	613.000	256.900	480	Meadow	400	2	0	0
Birchmatte	615.250	246.500	790	Pasture	5000	7	0.57	150
Bogental	617.550	246.750	900	Pasture	100	2	1.00	5
Gwidem	627.850	245.625	1000	Pasture	5625	337	–	–
Bälweid	629.375	253.075	530	Meadow	500	4	–	–

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 ($r_{27} = 0.74$, $P < 0.001$).

PLANT SIZE, AND OCCURRENCE AND SIZE OF POPULATIONS OF *M. REBELI*

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 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.

RELATIONSHIPS BETWEEN *G. CRUCIATA* POPULATION SIZE, GENET SIZE, FRUIT HERBIVORY AND PLANT REPRODUCTION

We randomly selected an additional 10–20 genets in each of our 1998 26 *G. cruciata* populations (procedure as above; one population was mown before fruits could be sampled) between 31 July and 14 August. For each genet, we counted the number of flower stems and, for one randomly selected stem per genet, measured its length and counted the number of fruits per stem. From each selected flower stem, we collected a random sample of 10 fruits. When the size of a population was smaller than 10, we sampled fruits from all genets, and when a stem carried less than 10 fruits, they were all collected. Each fruit was placed separately in a paper bag.

In the laboratory, we opened the fruits, checked them for the presence of fruit herbivores (indicated by partly eaten seeds, seeds woven together, or dead caterpillars) and counted the number of developed seeds per fruit. We multiplied this by the number of fruits per flower stem and the number of flower stems per genet to estimate the total number of developed seeds per genet.

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 χ^2-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 (Searle *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 *M. rebeli* with a 95% probability, we used Fieller's method (Collett 1991). The two *G. cruciata* populations located east of the current distribution of *M. rebeli* (Gonseth 1987) were 10.3 km from the nearest other study population and were not included in these analyses.

Variables were appropriately transformed if necessary to obtain normally distributed residuals. Population size was $\log_{10}$-transformed. For skewed distributions 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.; $t_{27} = -2.03$, $P = 0.052$). 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 ($t_{27} = -1.13$, $P = 0.269$) and altitude ($t_{27} = 1.08$, $P = 0.292$).

OCCURRENCE AND SIZE OF *M. REBELI* POPULATIONS

Overall, we recorded *M. rebeli* populations in 18 out of the 27 (66.6%) *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 $F_{1,25} = 26.86$, $P < 0.001$; 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 $F_{1,25} = 0.48$, $P = 0.37$), but was higher in pastures than in meadows (77.8 vs. 33.3%; approximate $F_{1,25} = 4.15$, $P = 0.052$). The effect of *G. cruciata* population size remained significant after eliminating the effect of habitat type (approximate $F_{1,24} = 26.46$, $P < 0.001$).

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, $F_{1,24} = 29.33$, $P < 0.001$; quadratic, $F_{1,24} = 9.07$, $P = 0.006$).

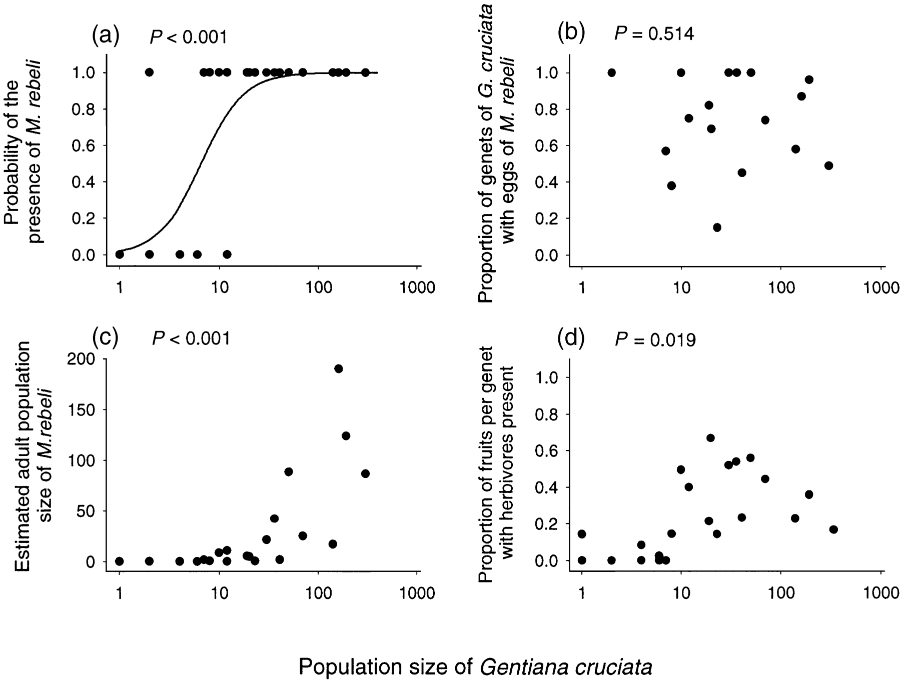

Fig. 1 The relationship between population size of *G. cruciata* and (a) the probability that a population of *M. rebeli* is present, (b) the proportion of genets of *G. cruciata* with eggs of *M. rebeli*, (c) the estimated adult population size of *M. rebeli*, and (d) the proportion of fruits per genet that were infested by herbivores. For significance tests, see text and Tables 2 and 4.

Table 2 (a) The effect of population size, plant size and year on the probability that the butterfly *M. rebeli* is present on individual genets of the plant *G. cruciata* ($n = 777$ genets). (b) The effect of population size and plant size on the number of *M. rebeli* eggs per flower stem ($\log_{10}$ transformed, $n = 255$ genets), and (c) the effect of population size and plant size on the estimated number of *M. rebeli* eggs per genet of *G. cruciata* ($\log_{10}(x + 1)$ transformed, $n = 254$ genets). Only those 18 *G. cruciata* populations that supported a butterfly population were analysed. The table is based on a generalized linear mixed model analysis for (a) and on a linear mixed model analysis for (b) and (c). Effects are fixed factor coefficients or random effect variance components in the linear model. For tests, see Methods

	(a) Presence of *M. rebeli* eggs on individual genets of *G. cruciata*			(b) Number of *M. rebeli* eggs per flower stem of *G. cruciata*			(c) Estimated number of *M. rebeli* eggs per genet of *G. cruciata*		
Source	Effect	χ^2_1	*p*	Effect	χ^2_1	*p*	Effect	χ^2_1	*p*
Population size of *G. cruciata*	0.175	0.4	0.514	0.170	1.1	0.294	0.328	2.7	0.100
Residual population variance component	1.857	323.0	< 0.001	0.177	84.8	< 0.001	0.275	88.6	< 0.001
Year (1999 vs. 1998)	0.039	0.6	0.453	–	–	–	–	–	–
Stem length of *G. cruciata*	0.070	34.6	< 0.001	0.020	21.1	< 0.001	0.036	44.3	< 0.001
Year × population size	0.253	0.5	0.497	–	–	–	–	–	–
Year × stem length	0.019	0.6	0.453	–	–	–	–	–	–
Residual genet variance component	0.613			0.202			0.329		

POPULATION AND GENET SIZE IN *G. CRUCIATA*

G. cruciata 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).

FRUIT HERBIVORY AND REPRODUCTION OF *G. CRUCIATA*

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,

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

Source	(a) No. flower stems per genet			(b) Stem length			(c) No. flowers per stem			(d) No. flowers per genet		
	Effect	χ^2_1	p	Effect	χ^2_1	p	Effect	χ^2_1	p	Effect	χ^2_1	p
Population size	0.039	2.0	0.157	−0.383	0.0	> 0.99	0.041	2.4	0.121	0.092	3.6	0.058
Residual population variance component	0.020	187.8	< 0.001	15.520	244.3	< 0.001	0.013	82.6	< 0.001	0.060	163.9	< 0.001
Year (1999 vs. 1998)	−0.031	5.4	0.020	1.708	9.1	0.003	0.112	27.2	< 0.001	0.083	4.2	0.040
Year × 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			0.176		

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)

Source	Effect	χ^2_1	p
Population size of *G. cruciata*	1.758	5.5	0.019
Presence of *M. rebeli* population (no-yes)	1.525	6.5	0.011
Population size of *G. cruciata* × presence of *M. rebeli* population	−0.746	0.0	> 0.99
Residual population variance component	0.720	18.5	< 0.001
Stem length of *G. cruciata*	0.113	0.1	0.752
Stem length of *G. cruciata* × presence of *M. rebeli* population	−0.115	3.1	0.078
Residual genet variance component	2.606		

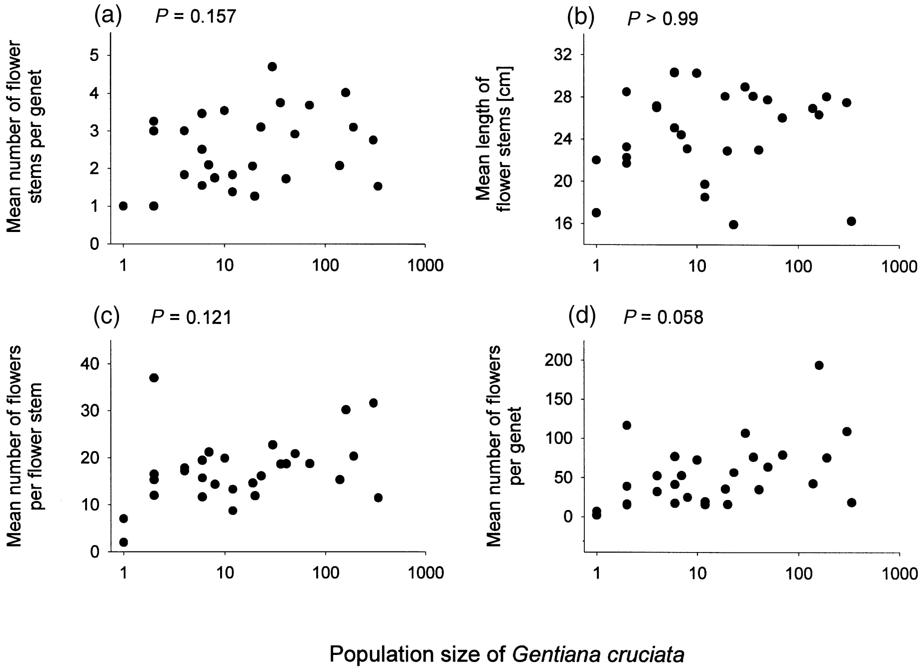

Fig. 2 The relationship between population size of *G. cruciata* and (a) the mean number of flower stems per genet, (b) the mean length of flower stems, (c) the mean number of flowers per flower stem, and (d) the mean number of flowers per genet. For significance tests, see Table 3.

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%; $t_{27} = 4.46$, $P < 0.001$; 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 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 *M. rebeli* population, as compared to 120 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, 2366 (qr: 926–6534), was independent of population size and of the presence of *M. rebeli* (Table 5b). The number of developed seeds per genet was greater where plants were larger (positive effect of stem height in Table 5b). However, there was also a greater number of *M. rebeli* eggs on these plants (Table 2c), resulting in an unexpected positive correlation at the genet level between the numbers of developed seeds and eggs (Table 5b). Our hypothesis that this relationship was due to an underlying third factor (plant size), which affected both numbers in the same way, was tested by introducing the number of flower stems per genet into the statistical model as a covariate. When size was controlled for in this way, the number of *M. rebeli* eggs per genet significantly reduced the number of seeds per genet, as expected from the results in Table 5a.

In summary, genets in larger populations of *G. cruciata* produced more fruits 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* for other herbivores (e.g. a moth: Forare & Solbreck 1997; 11 species of monophagous plant bugs, leaf hoppers and beetles: Zabel & Tscharntke 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 both 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, *Lycaeides 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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References

Ågren, J. (1996) Population size, pollinator limitation, and seed set in the self-compatible herb *Lythrum salicaria*. *Ecology*, **77**, 1779–1790.

Burdon, J.J., Ericson, L. & Müller, W.J. (1995) Temporal and spatial changes in a metapopulation of the rust pathogen *Triphragmium ulmariae* and its host, *Filipendula ulmaria*. *Journal of Ecology*, **83**, 979–989.

Breslow, N.E. & Clayton, D.G. (1993) Approximate inference in generalized linear mixed models. *Journal of the American Statistical Association*, **88**, 9–25.

Byers, D.L. (1995) Pollen quantity and quality as explanations for low seed set in small populations exemplified by *Eupatorium* (Asteraceae). *American Journal of Botany*, **82**, 1000–1006.

Carlsson, U. & Elmqvist, T. (1992) Epidemiology of anther-smut disease (*Microbotryum violaceum*) and numeric regulation of populations of *Silene dioica*. *Oecologia*, **90**, 509–517.

Carlsson-Granér, U., Elmqvist, T., Ågren, J., Gardfjell, H. & Ingvarsson, P. (1998) Floral sex ratios, disease and seed set in dioecious *Silene dioica*. *Journal of Ecology*, **86**, 79–91.

Collett, D. (1991) *Modelling Binary Data*. Chapman & Hall, London.

Dobson, A.J. (1990) *An Introduction to Generalized Linear Models*. Chapman & Hall, London.

Elmes, G.W., Clarke, R.T., Thomas, J.A. & Hochberg, M.E. (1996) Empirical tests of specific predictions made from a spatial model of the population dynamics of *Maculinea rebeli*, a parasitic butterfly of red ant colonies. *Acta Oecologica*, **17**, 61–80.

Ericson, L., Burdon, J.J. & Müller, W.J. (1999) Spatial and temporal dynamics of epidemics of the rust fungus *Uromyces valerianae* on populations of its host *Valeriana salina*. *Journal of Ecology*, **87**, 649–658.

Fischer, M. & Matthies, D. (1998a) The effect of population size on performance in the rare plant *Gentianella germanica*. *Journal of Ecology*, **86**, 195–204.

Fischer, M. & Matthies, D. (1998b) RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica*. *American Journal of Botany*, **85**, 811–819.

Fischer, M. & Stöcklin, J. (1997) Local extinctions of plants in remnants of extensively used calcareous grasslands 1950–85. *Conservation Biology*, **11**, 727–737.

Forare, J. & Solbreck, C. (1997) Population structure of a monophagous moth in a patchy landscape. *Ecological Entomology*, **22**, 256–263.

Gonseth, Y. (1987) *Verbreitungsatlas der Tagfalter der Schweiz (Lepidoptera Rhopalocera)*. Centre Suisse de Cartographie de la Faune, Neuchâtel.

Grevstad, F.S. (1996) Establishment of weed control agents under the influences of demographic stochasticity, environmental variability and Allee effects. *Proceedings of the IX International Symposium on Biological Control of Weeds* (eds V.C. Moran & J.H. Hoffmann), pp. 261–267. University of Cape Town, Stellenbosch.

Hegi, G. (1926) *Illustrierte Flora Von Mitteleuropa*, 3. Lehmanns, München.

Hill, J.K., Thomas, C.D. & Lewis, O.T. (1996) Effects of habitat patch size and isolation on dispersal by *Hesperia comma* butterflies: implications for metapopulation structure. *Journal of Animal Ecology*, **65**, 725–735.

Hochberg, M.E., Thomas, J.A. & Elmes, G.W. (1992) A modelling study of the population dynamics of a large blue butterfly, *Maculinea rebeli*, a parasite of red ant nests. *Journal of Animal Ecology*, **61**, 397–409.

Hochberg, M.E., Clarke, R.T., Elmes, G.W. & Thomas, J.A. (1994) Population dynamic consequences of direct and indirect interactions involving a large blue butterfly and its plant and red ant hosts. *Journal of Animal Ecology*, **63**, 375–391.

Hochberg, M.E., Elmes, G.W., Thomas, J.A. & Clarke, R.T. (1996) Mechanisms of local persistence in coupled host–parasitoid associations: the case model of *Maculinea rebeli* and *Ichneumon eumerus*. *Philosophical Transactions of the Royal Society of London B*, **351**, 1713–1724.

Kéry, M., Matthies, D. & Spillmann, H.-H. (2000) Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. *Journal of Ecology*, **88**, 17–30.

Keymer, R.J. & Leach, S.J. (1987) Calcareous grassland – a limited resource in Britain. *Calcareous grasslands – ecology and management. Proceedings of a BES/NCC Symposium at University of Sheffield, 1987* (eds S.H. Hillier, D.W.H. Walton & D.A. Wells), pp. 11–17. Bluntisham Books, Bluntisham.

Korneck, D., Schnittler, M. & Vollmer, I. (1996) Rote Liste der Farn- und Blütenpflanzen (Pteridophyta et Spermatophyta) Deutschlands. *Schriftenreihe für Vegetationskunde*, **28**, 21–187.

Knutson, R.L., Kwilosz, J.R. & Grundel, R. (1999) Movement patterns and population characteristics of the Karner blue butterfly (*Lycaeides melissa samuelis*) at Indiana Dunes National lakeshore. *Natural Areas Journal*, **33**, 109–120.

Landolt, E. (1991) *Gefährdung der Farn- und Blütenpflanzen in der Schweiz*. Bundesamt für Umwelt, Wald und Landschaft, Bern.

Lei, G.C. & Hanski, I. (1998) Spatial dynamics of two competing specialist parasitoids in a host metapopulation. *Journal of Animal Ecology*, **67**, 422–433.

Lepidopteren-Arbeitsgruppe (ed.) (1987) *Tagfalter und ihre Lebensräume*. Schweizerischer Bund für Naturschutz, Basel.

Littell, R.C., Milliken, G.A., Stroup, W.W. & Wolfinger, R.D. (1996) *SAS System for Mixed Models*. SAS Institute Inc., Cary, NC, USA.

McCullagh, P. & Nelder, J.A. (1989) *Generalized Linear Models*. Chapman & Hall, London.

Meusel, H., Jäger, E., Rauschert, S. & Weinert, E. (1978) *Vergleichende Chorologie der Zentraleuropäischen Flora*. Band II. Fischer-Verlag, Jena.

Morrell, C.H. (1998) Likelihood ratio testing of variance components in the linear mixed-effects model using restricted maximum likelihood. *Biometrics*, **54**, 1560–1568.

Oostermeijer, J.G.B., van Eijck, M.W. & den Nijs, J.C.M. (1994) Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae). *Oecologia*, **97**, 289–296.

Patterson, H.D. & Thompson, R. (1971) Recovery of inter-block information when block sizes are unequal. *Biometrika*, **58**, 545–554.

Payne, R.W., Lane, P.W., Digby, P.G.N., Harding, S.A., Leech, P.K., Morgan, G.W., Todd, A.D., Thompson, R., Tunnicliffe Wilson, G., Welham, S.J. & White, R.P. (1993) *Genstat 5, Release 3, Reference Manual*. Clarendon Press, Oxford.

Petanidou, T., den Nijs, J.C.M. & Oostermeijer, J.G.B. (1995) Pollination ecology and constraints on seed set of the rare perennial *Gentiana cruciata* L. in The Netherlands. *Acta Botanica Neerlandica*, **44**, 55–74.

Searle, S.R., Casella, G. & McCulloch, C.E. (1992) *Variance Components*. Wiley, New York.

Thomas, C.D. & Jones, T.M. (1993) Partial recovery of a skipper butterfly (*Hesperia comma*) from population refuges–lessons for conservation in a fragmented landscape. *Journal of Animal Ecology*, **62**, 472–481.

Thomas, J.A., Clarke, R.T., Elmes, G.W. & Hochberg, M.E. (1998) Population dynamics in the genus *Maculinea* (Lepidoptera: Lycaenidae). *Insect Populations* (eds J.P. Dempster & I.F.G. McLean), pp. 261–290. Kluwer Academic Publishers, Dordrecht.

Van Dyck, H., Oostermeijer, J.G.B., Talloen, W., Feenstra, V., van der Hidde, A. & Wynhoff, I. (2000) Does the presence of ant nests matter for oviposition to a specialized myrmecophilous *Maculinea* butterfly? *Proceedings of the Royal Society London, Series B*, **267**, 861–866.

Van Nouhuys, S. & Hanski, I. (1999) Host diet affects extinctions and colonizations in a parasitoid metapopulation. *Journal of Animal Ecology*, **68**, 1248–1258.

Waser, N.M. & Price, M.V. (1998) What plant ecologists can learn from zoology. *Perspectives in Plant Ecology, Evolution and Systematics*, **1/2**, 137–150.

Wells, S.M., Pyle, R.M. & Collins, N.M. (1983) *The IUCN Invertebrate Red Data Book*. IUCN, Gland, Switzerland.

Widén, B. (1993) Demographic and genetic effects on reproduction as related to population size in a rare, perennial herb, *Senecio integrifolius* (Asteraceae). *Biological Journal of the Linnean Society*, **50**, 179–195.

Young, A., Boyle, T. & Brown, T. (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution*, **11**, 413–418.

Zabel, J. & Tscharntke, T. (1998) Does fragmentation of *Urtica* habitats affect phytophagous and predatory insects differently? *Oecologia*, **116**, 419–425.

Zoller, H. & Wagner, C. (1986) Rückgang und Gefährdung von *Mesobromion*-Arten im Schweizer Jura. *Veröffentlichungen Des Geobotanischen Institutes der ETH, Stiftung Rübel*, **87**, 239–259.

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