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The genetic consequences of different dispersal behaviours in Lycaenid butterfly species

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Abstract

Many studies in population ecology have shown that related species have different dispersal behaviours. Species with sedentary and migratory behaviour exist in butterflies. While the genetic responses to population isolation are well studied, the effects of different dispersal behaviours of species are widely unknown. Therefore, we analysed 19 allozyme loci of two lycaenid butterflies, *Cupido minimus* as a sedentary butterfly and *Aricia agestis* as a mobile and expansive species. We collected 594 individuals (280 of *C. minimus* and 314 of *A. agestis*) in a western German study region with adjacent areas in Luxembourg and northeastern France. The genetic differentiation among populations of *A. agestis* ($F_{ST} = 3.9\%$) was lower than in *C. minimus* ($F_{ST} = 5.6\%$). Both species built up an isolation-by-distance system, which is more pronounced in *A. agestis* than in *C. minimus*. The genetic diversity in *C. minumus* populations (e.g. $P_{tot} = 73.5\%$) is higher for all analysed parameters than in *A. agestis* (e.g. $P_{tot} = 52.1\%$). Both species show specific genetic characteristics fitting with their different dispersal behaviours and respective ecological strategies. In the light of conservation genetics, we deduce that highly fragmented populations do not necessarily have a high extinction probability, but this risk depending much more on specific population genetic structures. In the studied species, *C. minimus* preserves a complex genetic constitution by high population densities. The patchily distributed *A. agestis* represents less rare alleles, present only in some populations, and holds up genetic diversity by high mobility.

Keywords: *Aricia agestis*, *Cupido minimus*, Lepidoptera, dispersal, allozyme electrophoresis, genetic diversity, conservation genetics

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Introduction

The theory of metapopuation ecology describes the presence of many organisms in a network of more or less interconnected local habitats (Hanski, 1991; Hanski & Gyllenberg, 1993). The survival probability of such a population network is determined by many factors like the ratio of habitat edge to interior (Chen *et al*., 1995; Radeloff *et al*., 2000), the isolation of habitat fragments (Collinge, 2000), patch area (Kruess & Tscharntke, 2000), patch quality (Dennis & Eales, 1997; Kuussaari *et al*., 2000; Hanski & Singer, 2001), microclimate (Braman *et al*., 2000) and the matrix between patches (Maes *et al*., 2004). All these factors contribute to determining the abundance of organisms in a landscape and, thus, influence a turnover equilibrium of colonisations, extinctions and recolonisations. However, habitat quality is of special importance for the persistence, principally of sendentary species, and, thus, their incidence probability in a patch (Dennis & Eales, 1997).

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Therefore, anthropogenic habitat fragmentation of previously continuous habitats has become a topic of growing interest in conservation biology (Wilcox & Murphy, 1985; Saunders *et al*., 1991; Frankham, 1995; Young *et al*., 1996) because isolation of large populations into several smaller and isolated populations may alter demographic and genetic factors, thereby increasing the risk of population extirpation (Goodman, 1987; Lacy, 1987; Harrison, 1991). Especially sedentary species with specific ecological requirements are affected by these changes because they occupy only part of their potential habitats as a consequence of habitat isolation (Dennis & Eales, 1997; Maes *et al*., 2004). One major goal of conservation genetic studies is, therefore, the analysis of the effects of such habitat fragmentations through the documentation of genetic differentiation among isolated populations and levels of genetic diversity within these populations (Harrisson & Hastings, 1996; Oostermeijer *et al*., 1996; Young *et al*., 1996). The effects of fragmentation are mostly determined by three key factors: (i) population density within patches; (ii) distances between patches (i.e. habitat availability); and (iii) dispersal ability of an organism. Population density and habitat availability depend on the ecological requirements (biotic and abiotic) of a species; dispersal behaviour influences the realised ecosystem connectivity of species (Holzhauer *et al*., 2005; Louy *et al*., 2007). Even in a group of mobile insects, such as butterflies, sedentary species are known with very restricted exchange rates between habitats (Conradt *et al*., 2001; Baguette, 2003; Vandewoestijne *et al*., 2004; Schmitt *et al*., 2006).

We hypothesize that species with high dispersal capacity, and thus high exchange rates among populations, show a continuous refreshment of their gene pool. On the other hand, colonisations of some few individuals often imply genetic bottlenecks and losses of rare alleles (Hedrick & Kalinowski, 2000; Keller & Waller, 2002; Reed & Frankham, 2003). Only some few main alleles persist, if such bottlenecks are repeated several times. Low exchange rates and high potential genetic drift within habitats will lead to a loss of genetic diversity in sedentary species. These processes may be counterbalanced by high population densities and low fluctuations, thus preserving a high genetic diversity.

To test the influence of different dispersal abilities and population densities of species living in fragmented environments, we selcted two lycaenid butterfly species with opposed ecological constraints. *Cupido minimus* (Fuessly, 1775) is one of the most sedentary buttterfly species of Europe (Weidemann, 1988; Bink, 1992; Cowley *et al*., 2001), building up high population densities. In contrast, *Aricia agestis* (Denis & Schiffermüller, 1775) is a much better disperser, and recently one of the most expansive butterfly species of Europe, but its population densities in general are considerably lower than in *C. minimus* (Bourn & Thomas, 1993; Asher *et al*., 2001; Lewis & Bryan, 2002). Individuals of *C. minimus* from seven sites and *A. agestis* from eight sites were analysed by allozyme electrophoresis.

As a study area, we chose the Rhineland-Palantinate and the Saarland (southwest Germany), including some adjoining areas in Luxembourg and northeastern France. With our study, we intend to answer the following questions:

i) Do the two butterfly species show remarkable differences in their population genetic structures within and among the populations analysed?

ii) Is the low dispersal power and the high population density of *C. minimus* reflected in its genetic structure, e.g. by a high genetic differentiation among populations, but a relatively high genetic diversity of the single populations?

iii) Is the higher dispersal power and the lower average density of *A. agestis* mirrored in its genetic structure, as well, for example, by only moderate genetic differentiations among populations, eventually with a remarkable isolation-by-distance structure but only a relatively low average of the genetic diversity of the single populations due to this limited census size?

Materials and methods

Study species

Cupido minimus is a sedentary butterfly species, which builds up very high densities and exists in mostly isolated populations. Inferred from censuses and the geography of populations, Weidemann (1988) and Bink (1992) reported very low migration rates for this species, and Cowley *et al*. (2001) ranked *C. minimus* as the most sedentary butterfly species in a comparison with 49 other British butterflies. *Anthyllis vulneraria* is the single food plant and main nectar source of *C. minimus*; and the species recently became mostly restricted to calcareous grasslands (Honnay *et al*., 2006), resulting in a patchy distribution (Ebert & Rennwald, 1991; Asher *et al*., 2001; Van Swaay, 2002).

Aricia agestis has high dispersal power and quickly spreads to newly emerging habitats. Therefore, the species recently has extended its distribution considerably, e.g. in the UK (Bourn & Thomas, 1993; Asher *et al*., 2001; Lewis & Bryant, 2002). In our study area, this species occurs on warm and dry slopes, meadows and fallow land (Ebert & Rennwald, 1991). The larvae feed on different *Geranium* species, *Erodium cicutarium* and *Helianthemum nummularium* in our study region (Settele *et al*., 1999). *Aricia agestis* shows lower intermediate densities (Bink, 1992). Thus, the two analysed species represent two contrary behaviours of dispersal and population biology.

Sampling

A total of 594 individuals of *A. agestis* and *C. minimus* were collected during the summers 2003 to 2005 in our study area (Rhineland-Palatinate and Saarland (western Germany) and adjacent regions of Luxembourg and northeastern France) (fig. 1) (314 individuals of *A. agestis* at eight sites, 280 of *C. minimus* at seven sites). Individuals were stored in liquid nitrogene until allozyme analysis. Sample sizes were 40 for each site of *C. minimus* and 38–40 individuals per site of *A. agestis*. We distinguished the sample locations in big and small habitats with 15 ha representing the threshold.

Allozyme analysis

Half of the abdomens of the imagos were homogenised in Pgm-buffer (Harris & Hopkinson, 1978) by ultrasound and centrifuged at 17,000 Xg for 5 min. The remaining tissue was stored for further analysis. We ran electrophoreses on cellulose acetate plates (Hebert & Beaton, 1993). We analysed 16 enzyme systems representing 19 loci for both species (for running conditions, see table 1).

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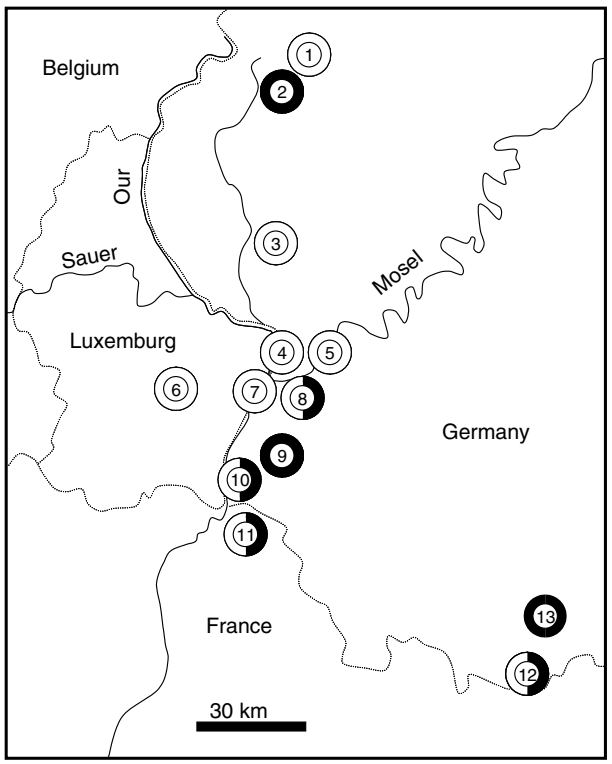

Fig. 1. The geographic location of the sample stations of *Cupido minimus* (black) and *Aricia agestis* (white) in Rhineland-Palatinate, Saarland, Loraine and Luxembourg. Dotted lines: country borders. 1, Lissendorf; 2, Weinsheim; 3, Ingendorf; 4, Igel; 5, Trier; 6, Niederanven; 7, Nittel; 8, Wasserliesch; 9, Freudenburg; 10, Perl; 11, Montenach; 12, Niedergailbach; 13, Mimbach.

Statistics

Alleles were labelled according to their relative mobility, starting with '1' for the slowest. The allele frequencies and genetic distances (Nei, 1978) were calculated with the package G-Stat (Siegismund, 1993). Hardy-Weinberg equilibrium (Louis & Dempster, 1987), genetic disequilibrium (Weir, 1991), locus by locus *F*-statistics and AMOVA variance analyses were calculated with the package Arlequin 2.000 (Schneider *et al*., 2000). Differences among means were analysed by Friedmann ANOVAs, Wilcoxon matched pairs tests or Mann-Whitney *U* tests using STATISTICA. Mantel tests were performed using X-Stat (http://www.xlstat.com/de/home/, July 2006).

Results

For *C. minimus*, 14 of the 19 loci analysed were polymorphic (see appendix) and only five loci had a single allele (Aat2, Fum, Gapdh, Acon, Hbdh). In *A. agestis*, only 11 of 19 loci analysed had two or more alleles (see appendix), whereas eight loci were monomorphic (6Pgdh, Idh2, Aat1, Gpdh, Fum, Gapdh, Acon, Apk). However, the total number of alleles detected in both species was quite similar, 49 in *A. agestis* and 47 in *C. minimus* (table 2). Both species also had similar mean numbers of alleles per locus

Table 1. Electrophoretic conditions for the different enzymes analysed for *Aricia agestis* and *Cupido minimus*. For each species, the following information is given: buffer, applications of homogenate and running time at 200 V.

locus	EC-Nr.	Number of loci	*Aricia agestis*	*Cupido minimus*
Pk	2.7.1.40	1	TC, 3, 30	TC, 3, 30
G6pdh	1.1.1.49	1	TC, 3, 40	TC, 3, 40
6Pgdh	1.1.1.44	1	TM, 3, 45	TM, 3, 45
Idh	1.1.1.42	2	TM, 3, 45	TM, 3, 45
Aat	2.6.1.1	2	TM, 4, 40	TC, 4, 40
Mdh	1.1.1.37	2	TC, 2, 40	TC, 2, 40
Pgi	5.3.1.9	1	TM, 1, 40	TM, 1, 40
Gpdh	1.1.1.8	1	TM, 4, 30	TM, 4, 30
Fum	4.2.1.2	1	TC, 3, 45	TC, 3, 45
Me	1.1.1.40	1	TC, 3, 40	TC, 3, 40
Gapdh	1.2.1.12	1	TC, 4, 40	TC, 4, 40
Acon	4.2.1.3	1	TC, 3, 45	TC, 3, 45
Hbdh	1.1.1.30	1	TG, 3, 30	TG, 4, 30
Apk	2.7.3.3	1	TG, 3, 30	TG, 3, 30
Pgm	5.4.2.2	1	TG, 3, 40	TG, 3, 40
Pep (Phe-Pro)	3.4.11/13	1	TG, 3, 25	TG, 4, 25

TC, Tris-citrate, pH = 8.2 (Richardson *et al*., 1986); TG, Tris-glycine pH = 8.5 (Hebert & Beaton, 1993); TM, Tris-maleic acid pH = 7.0 (adjusted from TM pH = 7.8 (Richardson *et al*., 1986)).

Table 2. Overview of detected alleles and their distribution pattern over the populations analysed of *Aricia agestis* and *Cupido minimus*.

	Aricia agestis	*Cupido minimus*
Total number of alleles	49	47
rare alleles*	18	19
not in all populations	19	19
in < 50% of the populations	12	12
in < 33% of the populations	5	11
max. number of alleles/locus	7	6
average percentage of alleles/site	82.2	90.6

*, overall frequency of less than 5%.

(*A. agestis*: 2.12 ± 0.10 SD, range 2.00–2.28; *C. minimus*: 2.24 ± 0.22 SD, range 1.93–2.64; Wilcoxon test: $P = 0.176$).

Rare alleles (fraction < 5%) were similarly common in *C. minimus* (19, i.e. 45.2% of all alleles) and *A. agestis* (18, i.e. 43.9% of all alleles). Nineteen alleles were not found in all populations of each of the two. Similar relations were found for alleles detected in less than half of the populations (*C. minimus*: 12, i.e. 28.6% of all alleles; *A. agestis*: 12, i.e. 29.3% of all alleles; Wilcoxon test: $P = 0.32$). However, alleles observed in less than 33% of all populations were significantly more common in *C. minimus* (11, i.e. 26.2% of all alleles) than in *A. agestis* (5, i.e. 12.2%; Wilcoxon text: $P < 0.001$). On average, 90.6% of all detected alleles were observed in a population of *C. minimus*, whereas 17.8% of all known alleles on average were missing in the *A. agestis* samples (table 2). The maximum number of alleles deteted per locus was higher in *A. agestis* (seven) than in *C. minimus* (six).

All further parameters of genetic diversity investigated were higher in *C. minimus* than in *A. agestis* (table 3). Except for P_{95}, these differences between both species were

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Table 3. Five parameters of genetic diversity for all populations analysed of *Aricia agestis* and *Cupido minimus*: mean number of alleles per locus (*A*), percentage of expected heterozygosity (H_e), percentage of observed heterozygosity (H_o), observed heterozygosity divided by the expected heterozygosity (H_o/H_e) percentage of loci with the most common allele not exceeding 95% (P_{95}) and total percentage of polymorphic loci (P_{tot}).

Site	area	*A*		H_e [%]		H_o [%]		H_o/H_e		P_{tot} [%]		P_{95} [%]	
		A. a.	*C. m*	*A. a.*	*C. m*	*A. a.*	*C. m*	*A. a.*	*C. m*	*A. a.*	*C. m*	*A. a.*	*C. m*
Lissendorf	e	2.00		15.4		10.5		0.68		44.4		33.3	
Weinsheim	e		2.29		18.8		18.8		1.00		78.6		50.0
Ingendorf	e	2.17		16.8		11.6		0.69		55.6		38.9	
Igel	m	2.22		16.7		12.0		0.72		55.6		38.9	
Trier	m	2.06		15.0		12.3		0.82		50.0		38.9	
Niederanven	m	2.06		15.4		12.7		0.82		55.6		44.4	
Nittel	m	2.00		13.6		13.4		0.99		50.0		33.3	
Wasserliesch	m		1.93		16.2		15.7		0.97		50.0		28.6
Freudenburg	m		2.14		19.6		19.3		0.98		64.3		42.9
Perl	m	2.28	2.36	14.5	19.7	11.9	18.5	0.82	0.94	55.6	85.7	38.9	50.0
Montenach	m		2.64		19.8		18.4		0.93		100.0		57.1
Niedergailbach	b	2.17	2.14	17.8	16.7	12.7	15.1	0.71	0.90	50.0	64.3	44.4	35.7
Mimbach	b		2.21		17.0		15.4		0.91		71.4		35.7
mean (±SD)		2.12 (±0.10)	2.24 (±0.22)	15.65 (±1.45)	18.26 (±1.57)	12.14 (±0.94)	17.31 (±1.82)	0.78 (±0.11)	0.95 (±0.04)	52.1 (±4.41)	73.47 (±16.35)	38.88 (±4.53)	42.86 (±10.10)
U test (*P*)		0.245		0.012		0.001		0.010		0.007		0.481	

e, Eifel; m, Mosel region; b, Bliesgau; *A.a., Aricia agestis; C. m., Cupido minimus*.
Tests for significant differences are performed using *U* test.

Table 4. Non-hierarchical variance analysis of the *Aricia agestis* and *Cupido minimus* populations analysed.

	A. agestis	*C. minimus*
among populations	0.057	0.075
within populations among individuals	0.315	0.062
within individuals	1.090	1.220
total variance	1.462	1.357
F_{ST} (%)	3.9	5.6
F_{IS} (%)	22.5	4.9

significant (H_e: *A. agestis*: 15.7% ± 1.5 SD, range 13.6–17.8%; *C. minimus*: 18.3% ± 1.6 SD, range 16.2–19.8%, *U* test: $P = 0.012$; H_o: *A. agestis*: 12.1% ± 0.9 SD, range 10.5–13.4%; *C. minimus*: 17.3% ± 1.8 SD, range 15.1–19.3%, *U* test: $P = 0.001$; P_{95}: *A. agestis*: 38.9 ± 4.5 SD, range 33.3% to 44.4%, *C. minimus*: 42.9% ± 10.1 SD, range 28.6–50.0%, U-test: $P = 0.481$; P_{tot}: *A. agestis*: of 52.1 ± 4.4 SD, range 44.4–55.6%; *C. minimus* 73.5% ± 16.4 SD, range 50.0–100.0%, *U* test: $P = 0.007$).

Remarkable deviations from Hardy-Weinberg equilibrium were detected after Bonferroni correction (*A. agestis*: G6pdh, Mdh1, Mdh2, Me in Perl (D), G6pdh, Aat2, Hbdh, Mdh1, Me, Pgm in Niedergailbach (D), G6pdh, Pgi, Hbdh, Me, Pgm in Igel (D), G6pdh, Aat2, Mdh2, Me in Lissendorf (D), G6pdh, Hbdh, Mdh2 in Niederanven (L), Me in Nittel (D) and G6pdh, Hbdh, Idh1, Mdh1 and Me in Ingendorf (D); C. *minimus*: Pgi and Pep in Wasserliesch (D), G6pdh in Badstube and Pep in Perl (D)). While 28 deviations from Hardy Weinberg equilibrium were detected for *A. agestis*, only four cases were found for *C. minimus*. No significant linkage disequilibrium was detected for any pair of loci, an expected result in species with about 24 chromosome pairs (Fernández-Rubio, 1991).

All analysed parameters of genetic diversity were not significantly different between populations thriving on big and small patches in both species (*U* test: all $P > 0.05$).

The total genetic variance among populations and within individuals was higher in *C. minimus* than in *A. agestis* (see table 4). The relative differentiation among populations of *A. agestis* ($F_{ST} = 3.9\%$, $P < 0.001$) was also lower than in *C. minimus* ($F_{ST} = 5.6\%$, $P < 0.001$). The F_{IS} value was high for *A. agestis* ($F_{IS} = 22.5\%$, $P = 0.001$) and comparatively low for *C. minimus* ($F_{IS} = 4.9\%$, $P < 0.001$).

The mean of the genetic distances (Nei, 1978) between *C. minimus* samples (0.0304 ± 0.0103 SD) was higher than in *A. agestis* (0.0232 ± 0.0096 SD). These means were marginally significantly different (*U* test, $P = 0.06$). A significant isolation-by-distance system was detected in both species; this correlation between geographic and genetic distances was stronger in *A. agestis* ($r^2 = 0.318$; Mantel test: $P = 0.003$) than in *C. minimus* ($r^2 = 0.218$, Mantel test: $P = 0.031$) (fig. 2).

Discussion

The genetic diversity for all parameters analysed of the studied populations of *C. minimus* was higher than in *A. agestis* with most of the differences being significant. The parameters of the genetic diversity of both species are at an upper intermediate level in comparison with other butterfly and moth species (Porter & Geiger, 1988; Porter & Shapiro, 1989; Descimon, 1995; Pelz, 1995; Johannesen *et al*., 1996, 1997; Meglecz *et al*., 1997; Schmitt & Seitz, 2001a; Habel *et al*.,

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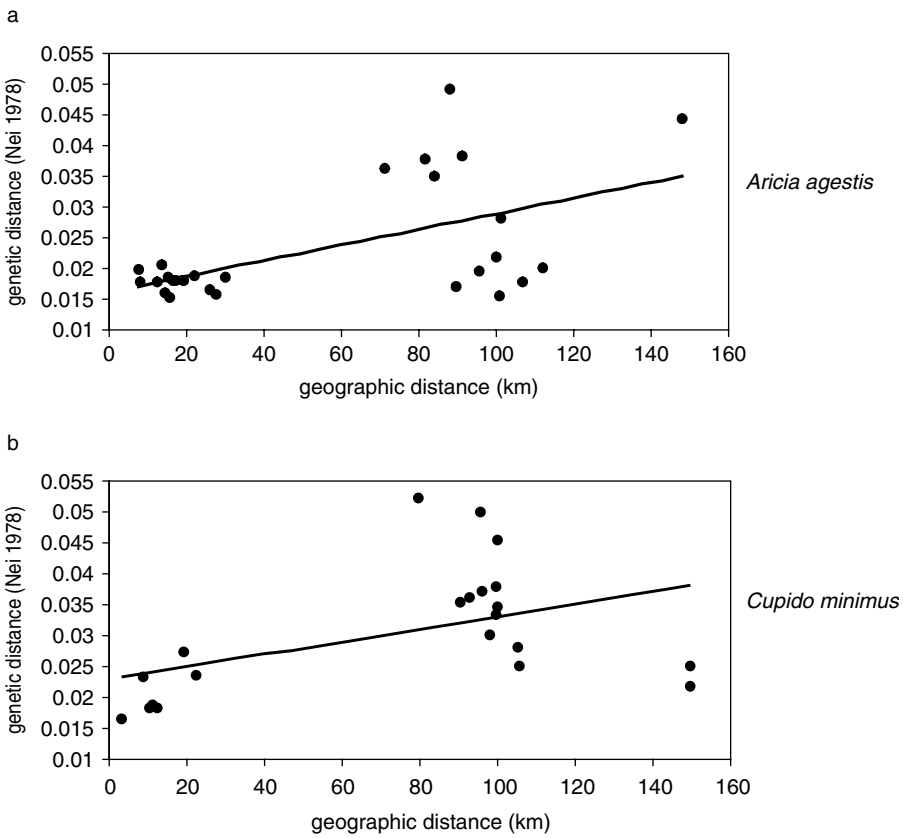

Fig. 2. Correlation between the geographic distances and the respective genetic distances (Nei, 1978) of the populations of (a) *Aricia agestis* ($r^2 = 0.318$; Mantel test: $P = 0.003$) and (b) *Cupido minimus* ($r^2 = 0.218$, Mantel test: $P = 0.031$).

Table 5. Overview of the parameters of genetic diversity and F_{ST} for four Lycaenidae in our study area.

Parameter	*Aricia agestis*	*Cupido minimus*	*Polyommatus coridon*	*Polyommatus icarus*
A	2.12 (±0.10)	2.24 (±0.22)	2.54 (±0.16)	2.94 (±0.22)
H_e [%]	15.7 (±1.5)	18.3 (±1.6)	19.6 (±1.5)	17.7 (±0.7)
P_{95} [%]	38.9 (±4.5)	42.9 (±10.1)	54.0 (±6.4)	46.3 (±5.6)
P_{tot} [%]	52.1 (±4.4)	73.5 (±16.4)	73.8 (±7.4)	80.3 (±7.1)
F_{ST} [%]	3.92 ($P = 0.001$)	5.67 ($P = 0.001$)	1.43 ($P < 0.001$)	0.41 ($P = 0.99$)
N (populations)	8	7	22	15

For abbreviations, see table 3.

2005; Schmitt *et al*., 2005). However, genetic diversities tend to be rather high in lycaenid butterflies (Peterson, 1995; Brookes *et al*., 1997; Schmitt & Seitz, 2001b; Schmitt *et al*., 2003), and only some rare and localised species of this family show considerably lower values than in our case (Gadeberg & Boomsma, 1997; Packer *et al*., 1998; Figurny-Puchalska *et al*., 2000; Bereczki *et al*., 2005). Two other lycaenid butterflies analysed in the same study area (*Polyommatus coridon*: Schmitt & Seitz, 2002; Schmitt *et al*., 2002; *Polyommatus icarus*: Schmitt *et al*., 2003; both data sets adapted to the study area of this study), in general, show considerably higher values of their parameters of genetic diversity than *C. minimus* and *A. agestis* (table 5).

The genetic differentiation among the analysed *C. minimus* samples was comparatively high. The F_{ST} value of 5.6% was in the order of magnitude or even higher than in common species on the continental scale (Porter & Geiger, 1995; Schmitt & Seitz, 2001b; Schmitt *et al*., 2003, 2005; Habel *et al*., 2005). Another habitat specialist butterfly, the skipper *Thymelicus acteon*, showed a quite similar value in the same study area (Louy *et al*., 2007), whereas two common skipper species (*T. sylvestris* and *T. lineola*) and the two lycaenid species, *P. coridon* and *P. icarus* (Schmitt & Seitz, 2001b; Schmitt *et al*., 2002, 2003: table 5), had much less of their genetic variance distributed among populations.

The F_{ST} value calculated for *A. agestis* is somewhat lower than in *C. minimus* but, nevertheless, higher than in the two other lycaenid species analysed in the same study area. However, the F_{IS} of this species (22.5%) is considerably higher than in all the other studies mentioned above.

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These values of genetic diversity of the populations and differentiation among populations reflect the population ecology and distribution patterns of both species in the study area. *Cupido minimus* is known as a very sedentary species (Weidemann, 1988; Bink, 1992; Baguette *et al*., 2000), ranked as the most sedentary butterfly of the UK (Cowley *et al*., 2001). As the species is restricted to semi-natural calcareous grasslands in our study area, a strong restriction of gene-flow among the mostly isolated patches would seem to be the logical consequence, generating the observed high F_{ST} value. Although *A. agestis* is also restricted to isolated habitat patches of extensively managed grasslands, the dispersal ability of this species is considerably higher (Bink, 1992; Cowley *et al*., 2001; Thomas *et al*., 2001), thus explaining the lower genetic differentiation among populations. Interestingly, *A. agestis* exhibits considerably higher genetic differentiation among populations ($F_{ST} = 9.3\%$) in a regional study in the UK, where the habitat fragmentation is more pronounced than in our study area, thus leading to less exchange of individuals (Wynne *et al*., 2008). The lack of genetic differentiation among populations of the widespread habitat generalist, *P. icarus*, in our study area (Schmitt *et al*., 2003) fits with the genetic differentiation of the other lycaenid species analysed, as well as the low or missing differentiation in the two generalist skipper species, *T. sylvestris* and *T. lineola*, respectively (Louy *et al*., 2007).

However, the rather low F_{ST} value of *P. coridon* in the study area (1.4%) seems contradictory because this species is strongly restricted to semi-natural calcareous grasslands (Ebert & Rennwald, 1991; Asher *et al*., 2001) and only moderately mobile but with occasional long distance dispersal (Ebert & Rennwald, 1991; Asher *et al*., 2001; Cowley *et al*., 2001). However, the population densities and total numbers of individuals per population, in general, strongly exceed the ones observed, e.g. in *A. agestis* (Bink, 1992). Therefore, genetic bottlenecks might be rare in *P. coridon* and occasional exchange of individuals between populations might not notably influence their genetic texture, thus not leading to an isolation-by-distance equilibrium, neither in our study area (Schmitt & Seitz, 2002) nor in a study in Lower Saxony (Krauss *et al*., 2004a).

In *A. agestis*, the relatively low mean population densities make this species prone to population bottlenecks and, thus, enhance the importance of immigration of individuals on the genetic texture of each single population. These ecological constraints might explain the evolution of a pronounced isolation-by-distance equilibrium over our study area, explaining about 32% of the genetic differentiation among populations. As population densities of *C. minimus* are, on average, considerably higher than in *A. agestis* and exchange of individuals between populations is much less, the isolation-by-distance system is less pronounced in *C. minimus*.

The ecological demands of these four lycaenid butterflies analysed in our study area are also mirrored in their genetic diversities; the lowest average genetic richness of the populations was found in *A. agestis*, the species with the lowest population densities and most probably frequent genetic bottlenecks. Such bottlenecks are further supported by the relatively low average percentage of alleles per population compared to all detected alleles (82%) being considerably lower than in *C. miminus* (91%), which most probably is less prone to bottlenecks. Apparently, the populations of the latter species are so large and stable that most of the populations' genetic diversity is preserved over time; a similar situation with even higher population densities and higher genetic richness was found in *P. coridon* (Schmitt & Seitz, 2002). In contrast, the total genetic diversity observed in *A. agestis* is not maintained within the single populations but by the interaction among populations in a metapopulation system; a somewhat similar situation, but in a widely distributed generalist species, might exist in the case of *P. icarus* (Schmitt *et al*., 2003).

Conservation implications

Our results have a positive message for nature conservation in our study area. The genetic data obtained for the weakly dispersing *C. minimus* point out that this species is not vitally dependent upon an intact metapopulation structure. Rather, each population analysed seems to have a high survival probability based on the high genetic diversities found in all of them, and deleterious effects due to genetic depressions are little likely. Therefore, the conservation of its habitats is crucial for the survival of this species; here, the habitat quality is the driving force for the number of observed individuals and not the geographical extension. The density of the larval food plant, *Anthyllis vulneraria*, is of outstanding importance in the case of *C. minimus* (León-Cortés *et al*., 2003; Krauss *et al*., 2004b). The connectivity between habitats (and thus the exchange of single individuals) seems to be of less importance. Similar situations have been observed in other sedentary species, mostly based on ecological data sets (e.g. *Hesperia comma*, *T. acteon* and *Mellicta athalia*: Thomas *et al*., 2001; *Coenonympha tullia*: Dennis & Eales, 1997; *Melitaea cinxia*: Hanski, 1999; *Polyommatus coridon*: Schmitt *et al*., 2006; *Maculinea alcon*: Wallis DeVries, 2004, Habel *et al*., 2007).

The situation of *A. agestis* is strongly divergent from the one of *C. minumus* because of the existence of an intact metapopulation structure with an extinction-recolonisation cycle, and a strong gene-flow among habitats seems to be vital for this species (Habel, personal observations; Wynne *et al*., 2008). This is underlined by the existence of a pronounced isolation-by-distance equilibrium in this species. Therefore, habitat quality itself and the geographical extension of patches apparently play a minor role. Similar situations are commonly observed in other species (Hanski, 1994; Nève *et al*., 1996; Mousson *et al*., 1999).

The combination of these two opposed strategies warns against untroubled optimism because conservation strategies focused on one of these two groups distinguished above will have detrimental consequences for the other one. Further, several species follow one of the scenarios in one landscape, but seem to be completely adapted to the other one in other parts of the species' distribution area (e.g. *Maculinea* species: Wynhoff, 2001), thus making the definition of clear conservation guidelines even for single species a difficult task, if aimed on an interregional scale. Therefore, the combination of (i) a strict conservation of the existing habitats maintaining their quality for the species in focus on the one hand and (ii) a well elaborated conservation plan for the sustainment of habitat interconnections and metapopulations including the preservation of multiple stepping stone habitats on the other hand will be crucial to preserve the biodiversity resources of Central European agricultural landscapes with all their complexity.

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References

Asher, J., Warren, M., Fox, R., Harding, P., Jeffcoate, G. & Jeffcoate, S. (2001) *The Millennium Atlas of Butterflies in Britain and Ireland*. 456 pp. Oxford, UK, Oxford University Press.

Baguette, M. (2003) Long distance dispersal and landscape occupancy in a metapopuation of the cranberry fritillary butterfly. *Ecography* **26**, 153–160.

Baguette, M., Petit, S. & Quéva, F. (2000) Population spatial structure and migration of three butterfly species within the same habitat network: consequences for conservation. *Journal of Applied Ecology* **37**, 100–108.

Bereczki, J., Pecsenye, K., Peregovits, L. & Varga, Z. (2005) Pattern of genetic differentiation in the *Maculinea alcon* species group (Lepidoptera, Lycaenidae) in Central Europe. *Journal of Zoological Systematics and Evolution Research* **43**, 157–165.

Bink, F.A. (1992) *Ecologische Atlas van de Dagvlinders van Noordwest-Europa*. 512 pp. Haarlem, The Netherlands, Schuyt & Co., Uitgevers en Importeurs.

Bourn, N.A. de & Thomas, J.A. (1993) The ecology and conservation of the brown argus butterfly *Aricia agestis* in Britain. *Biological Conservation* **63**, 67–74.

Braman, S.K., Latimer, J.G., Oetting, R.D., McQueen, R.D., Eckberg, T.B. & Prinster, M. (2000) Management strategy, shade and landscape composition effects on urban landscape plant quality and arthropod abundance. *Journal of Economic Entomology* **93**, 1464–1472.

Brookes, M.I., Graneau, Y.A., King, P., Rose, O.C., Thomas, C.D. & Mallet, J.L.B. (1997) Genetic analysis of founder bottlenecks in the rare british butterfly *Plebejus argus*. *Conservation Biology* **11**, 648–661.

Chen, J., Franklin, J.F. & Spies, T.A. (1995) Growing-season microclimatic gradients from clearcut edges into old-growth Douglas-fir forests. *Ecological Applications* **5**, 74–86.

Collinge, S.K. (2000) Effects of grassland fragmentation on insect species loss, colonization and movement patterns. *Ecology* **81**, 66–84.

Conradt, L., Roper, T.J. & Thomas, C.D. (2001) Dispersal behaiour of individuals in metapopulations of two British butterflies. *Oikos* **95**, 416–424.

Cowley, M.J.R., Thomas, C.D., Roy, D.B., Wilson, R.J., Léon-Cortés, J.L., Guitiérrez, D., Bulman, C.R., Quinn, R.M., Moss, D. & Gastzzon, K.J. (2001) Density–distribution relationships in British butterflies, the effect of mobility and spatial scale. *Journal of Animal Ecology* **70**, 410–425.

Dennis, R.L.H. & Eales., H. (1997) Patch occupancy in *Coenonympha tullia* (Müller 1764) (Lepidoptera: Satyrinae): Habitat quality matters as much as patch size and isolation. *Journal of Insect Conservation* **1**, 167–176.

Descimon, H. (1995) La conservation des *Parnassius* en France: aspects zoogéographiques, écologiques, démographiques et génétiques. *Editions OPIE* **1**, 1–54.

Ebert, G. & Rennwald, E. (*Ed.*) (1991) *Die Schmetterlinge Baden-Württembergs, Vol. 2*. 535 pp. Stuttgart, Germany, Eugen Ulmer.

Fernández-Rubio, F. (1991) *Guia de mariposas diurnas de la Peninsular Ibérica, Baleares, Canarias, Azores y Madeira (Libytheidae, Nymphalidae, Riodinidae y Lycaenidae)*. Madrid, Spain, Ediciones Pirámide.

Figurny-Puchalska, E., Gadeberg, R.M.E. & Boomsma, J.J. (2000) Comparison of genetic population structure of the large blue butterfly *Maculinea nausithous* and *M. teleius*. *Biodiversity and Conservation* **9**, 419–432.

Frankham, R. (1995) Conservation genetics. *Annual Reviews in Genetics* **29**, 305–327.

Gadeberg, R.M.E. & Boomsma, J.J. (1997) Genetic population structure of the large blue butterfly *Maculinea alcon* in Denmark. *Journal of Insect Conservation* **1**, 99–111.

Goodman, D. (1987) The demography of change extinction. pp. 11–43 *in* Soulé, M.E. (*Ed.*) *Viable Populations for Conservation*. Cambridge, UK, Cambridge University Press.

Habel, J.C., Schmitt, T. & Müller, P. (2005) The fourth paradigm pattern of postglacial range expansion of European terrestrial species: The phylogeography of the Marbled White butterfly (Satyrinae, Lepidoptera). *Journal of Biogeography* **32**, 1489–1497.

Habel, J.C., Schmitt, T., Härdtle, W., Lütkepohl, M. & Assmann, T (2007) Dynamics in a butterfly-plant-ant system: influence of habitat characteristics on turnover rates of the endangered lycaenid *Maculinea alcon*. *Ecological Entomology* **32**, 536–543.

Hanski, I. (1991) Single species metapopulation dynamics: concepts, models, and observations. *Biological Journal of the Linnean Society* **42**, 17–38.

Hanski, I. (1994) Patch-occupancy dynamics in fragmented landscapes. *Trends in Ecology and Evolution* **9**, 131–135.

Hanski, I. (1999) *Metapopulation Ecology*. 313 pp. Oxford, UK, Oxford University Press.

Hanski, I. & Gyllenberg, M. (1993) Two general metapopulation models and the corre-satellite species hypothesis. *Nature* **142**, 17–41.

Hanski, I. & Singer, M.C. (2001) Extinction, colonization dynamics and host-plant choice in butterfly metapopulations. *American Naturalist* **158**, 341–353.

Harris, H. & Hopkinson, D.A. (1978) *Handbook of Enzyme Electrophoresis in Human Genetics*. 310 pp. Amsterdam, The Netherlands, University of Amsterdam.

Harrison, S. (1991) Local extinction in a metapopulation context: an empirical evaluation. *Biologial Journal of the Linean Society* **42**, 73–88.

Harrison, S. & Hastings, A. (1996) Genetic and evolutionary consequences of metapopulation structure. *Trends in Ecology and Evolution* **11**, 180–183.

Hebert, P.D.N. & Beaton, M.J. (1993) *Methodologies for Allozyme Analysis using Cellulose Acetat Electrophoresis*. 43 pp. Beaumont, Texas, USA, Helena Laboratories.

Hedrick, P.W. & Kalinowski., S.T. (2000) Inbreeding Depression in Conservation Biology. *Annual Review of Ecology and Systematics* **31**, 139–162.

Holzhauer, S.I.J., Ekschmitt, K., Sanders, A.-C., Dauber, J. & Wolters, V. (2005) Effect of historic landscape change on

the genetic structure of the bush-cricket *Metrioptera roeseli*. *Landscape Ecology* **34**, 23–35.

Honnay, O., Coart, E., Butaye, J., Adriaens, D., Van Glabeke, S. & Roldán-Ruiz, I. (2006) Low impact of present and historical landscape configuration on the genetics of fragmented *Anthyllis vulneraria* populations. *Biological Conservation* **127**, 411–419.

Johannesen, J., Schwing, U., Seufert, W., Seitz, A. & Veith, M. (1997) Analysis of gene flow and habitat patch network for *Chazara briseis* (Lepidoptera: Satyridae) in an agricultural landscape. *Biochemical Systematics and Ecology* **25**, 419–427.

Johannesen, J., Veith, M. & Seitz, A. (1996) Population genetic structure of the butterfly *Melitaea didyma* (Nymphalidae) along a northern distribution range border. *Molecular Ecology* **5**, 259–267.

Keller, L.F. & Waller, D.M. (2002) Inbreededing effects in wild populations. *Trends in Ecology and Evolution* **17**, 230–241.

Krauss, J., Schmitt, T., Seitz, A., Steffan-Dewenter, I. & Tscharntke, T. (2004a) Effects of habitat fragmentation on the genetic structure of the monophagous butterfly *Polyommatus coridon* along its northern range margin. *Molecular Ecology* **13**, 311–320.

Krauss, J., Steffan-Dewenter, I. & Tscharntke, T. (2004b) Landscape occupancy and local population size depends on host plant distribution in the butterfly *Cupido minimus*. *Biological Conservation* **120**, 355–361.

Kruess, A. & Tscharntke, T. (2000) Species richness and parasitism in a fragmented landscape: experiments and field studies with insects on *Vicia sepium*. *Oecologia* **122**, 129–137.

Kuussaari, M., Hanski, I. & Singer, M. (2000) Local speciation and landscape-level influence on host use in an herbivorous insect. *Ecology* **81**, 2177–2187.

Lacy, R.C. (1987) Loss of genetic diversity from managed populations: Interacting effects of drift, mutation, immigration, selection and population subdivision. *Conservation Biology* **1**, 143–158.

León-Cortés, J.L., Lennon, J.J. & Tomas, C.D. (2003) Ecological dynamics of extinct species in empty habitat networks, 2, The role of host plant dynamics. *Oikos* **102**, 465–477.

Lewis, O.T. & Bryant, S.R. (2002) Butterflies on the move. *Trends in Ecology and Evolution* **17**, 351–352.

Louis, E.J. & Dempster, E.R. (1987) An exact test for Hardy-Weinberg and multiple alleles. *Biometrics* **43**, 805–811.

Louy, D., Habel, J.C., Schmitt, T., Assmann, T., Meyer, M. & Müller, P. (2007) Strongly diverging population genetic patterns of three skipper species: isolation, restricted gene flow and panmixis. *Conservation Genetics* **8**, 671–681.

Maes, D., Vanreuse, W., Talloen, W. & Van Dyck, H. (2004) Functional conservation units for the endangered Alcon Blue butterfly *Maculinea alcon* in Belgium (Lepidoptera: Lycaenidae). *Biological Conservation* **120**, 229–241.

Meglecz, E., Pecsenye, K., Peregovits, L. & Varga, Z. (1997) Allozyme variation in *Parnassius mnemosyne* (L.) (Lepidoptera) populations in North-East Hungary: variation within a subspecies group. *Genetica* **101**, 59–66.

Mousson, L., Nève, G. & Baguette, M. (1999) Metapopulation structure and conservation of the the dramberry fritillary *Boloria aquilonaris* (Lepidoptera, Nymphalidae) in Belgium. *Biological Conservation* **87**, 285–293.

Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.

Nève, G., Barascud, B., Hughes, R., Aubert, J., Descimon, H., Lebrun, P. & Baguette, M. (1996) Dispersal, colonization power and metapopulation structure in the vulnerable butterfly *Proclossiana eunomia* (Lepidoptera: Nymphalidae). *Journal of Applied Ecology* **33**, 14–22.

Oostermeijer, J.G.B., Brugman, M.L., Den Boer, E.R. & Den Nijs, H.C.M. (1996) Temporal and spatial variation in the demography of *Gentiana pneumonanthe*, a rare perennial herb. *Journal of Ecology* **84**, 166–174.

Packer, L., Taylor, J.S., Savignano, D.A., Bleser, C.A., Lane, C.P. & Sommers, L.A. (1998) Population biology of an endangered butterfly, *Lycaeides melissa samuelis* (Lepidoptera; Lycaenidae): genetic variation, gene flow and taxonomic status. *Canadian Journal of Zoology* **76**, 320–329.

Pelz, V. (1995) Biosystematik der europäischen Arten des Tribus Melitaeini Newman, 1870. *Oedippus* **11**, 1–62.

Peterson, M.A. (1995) Phenological isolation, gene flow and developmental differences among low- and high-elevation populations of *Euphilotes enoptes* (Lepidoptera: Lycaenidae). *Evolution* **49**, 446–455.

Porter, A.H. & Geiger, H. (1988) Genetic and phenotypic population structure of the *Coenonympha tullia* complex (Lepidoptera: Nymphalidae: Satyrinae) in California: no evidence for species boundaries. *Canandian Journal of Zoology* **66**, 2751–2765.

Porter, A.H. & Geiger, H. (1995) Limitations to the inference of gene flow at regional geographic scales – an example from the *Pieris napi* group (Lepidoptera: Pieridae) in Europe. *Biological Journal of the Linnean Society* **54**, 329–348.

Porter, A.H. & Shapiro, A.M. (1989) Genetics and Biogeography of the *Oeneis chryxus* Complex (Satyrinae) in California. *The Journal of Research on the Lepidoptera* **28**, 263–276.

Radeloff, V.C., Mladenoff, D.J. & Boyce, M.S. (2000) The changing relation of landscape patterns and jack pine budworm populations during an outbreak. *Oikos* **90**, 417–430.

Reed, D.H. & Frankham, R. (2003) Correlation between fitness and genetic diversity. *Conservation Biology* **17**, 230–237.

Richardson, B.J., Baverstock, P.R. & Adams, M. (1986) *Allozyme Electrophoresis: A Handbook for Animal Systematics and Population Studies*. 420 pp. San Diego, CA, Academic Press.

Saunders, D.A., Hobbs, R.J. & Margules, C.R. (1991) Biological consequences of ecosystem fragmentation: a review. *Conservation Biology* **5**, 18–32.

Schmitt, T. & Seitz, A. (2001a) Intraspecific allozymatic differentiation reveals the glacial refugia and the postglacial expansions of European *Erebia medusa* (Lepidoptera: Nymphalidae). *Biological Journal of the Linnean Society* **74**, 429–458.

Schmitt, T. & Seitz, A. (2001b) Allozyme variation in *Polyommatus coridon* (Lepidoptera: Lycaenidae): identification of ice-age refugia and reconstruction of post-glacial expansion. *Journal of Biogeography* **28**, 1129–1136.

Schmitt, T. & Seitz, A. (2002) Influence of habitat fragmentation on the genetic structure of *Polyommatus coridon* (Lepidoptera: Lycaenidae): implications for conservation. *Biological Conservation* **107**, 291–297.

Schmitt, T., Gießl, A. & Seitz, A. (2002) Postglacial colonisation of western Central Europe by *Polyommatus coridon* (Poda 1761) (Lepidoptera: Lycaenidae): evidence from population genetics. *Heredity* **88**, 26–34.

Schmitt, T., Gießl, A. & Seitz, A. (2003) Did *Polyommatus icarus* (Lepidoptera: Lycaenidae) have distinct glacial refugia in

southern Europe? – Evidence from population genetics. *Biological Journal of the Linnean Society* **80**, 529–538.

Schmitt, T., Röber, S. & Seitz, A. (2005) Is the last glaciation the only relevant event for the present genetic population structure of the Meadow Brown butterfly *Maniola jurtina* (Lepidoptera: Nymphalidae)? *Biological Journal of the Linnean Society* **85**, 419–431.

Schmitt, T., Habel, J.C., Besold, J., Becker, T., Johnen, L., Knolle, M., Rzepecki, A., Schultze, J. & Zapp, A. (2006) The Chalk-hill Blue *Polyommatus coridon* (Lycaenidae, Lepidoptera) in a highly fragmented landscape: How sedentary is a sedentary butterfly? *Journal of Insect Conservation* **10**, 311–316.

Schneider, S., Roessli, D. & Excoffier, L. (2000) Arlequin ver. 2.000 – A software for population genetics data analysis. Genève, Switzerland, Anthropology, University of Genève.

Settele, J., Feldmann, R. & Reinhardt, R. (1999) *Die Tagfalter Deutschlands – Ein Handbuch für Freilandökologen, Umweltplaner und Naturschützer*. 452 pp. Stuttgart, Germany, Ulmer.

Siegismund, H.R. (1993) G-Stat, ver. 3, Genetical statistical programs for the analysis of population data. The Arboretum, Royal Veterinary and Agricultural University, Denmark.

Thomas, J.A., Bourn, N.A.D., Clarke, R.T., Stewart, K.E., Simcox, D.J., Pearman, G.S., Curtis, R. & Goodger, B. (2001) The quality and isolation of habitat patches both determine where butterflies persist in fragmented landscapes. *Proceedings of the Royal Society of London, Series B* **268**, 1791–1796.

Vandewoestijne, S., Martin, T., Liégeois, S. & Baguette, M. (2004) Dispersal, landscape occupnacy and popualtion structure in the butterfly *Melanargia galathea*. *Basic and Applied Ecology* **5**, 581–591.

Van Swaay, C.A.M. (2002) The importance of calcareous grasslands for butterflies in Europe. *Biological Conservation* **104**, 315–318.

Wallis DeVries, M.F. (2004) From habitat quality assessment to conservation measures: a quantitative approach for the endangered butterfly *Maculinea alcon*. *Conservation Biology* **18**, 489–499.

Weidemann, H.-J. (1988) Tagfalter, Band 2. 372 pp. Melsungen, Germany, Verlag J. Neumann-Neudamm.

Weir, B.S. (1991) *Genetic Data Analysis*. 400 pp. Sunderland, MA, USA, Sinauer.

Wilcox, B.A. & Murphy, D.D. (1985) Conservation strategy: the effects of fragmentation on extinction. *American Naturalist* **125**, 879–887.

Wynhoff, I. (2001) At home on foreign meadows, the reintroduction of two *Maculinea* butterfly species. PhD thesis, Wageningen Agricultural University, The Netherlands.

Wynne, I.R., Wilson, R.J., Burke, A.S., Simpson, F., Pullin, A.S., Thomas, C.D. & Mallet, J. (2008) The effect of metapopulation processes on the spatial scale of adaptation in *Aricia* butterflies across an environmental gradient. Available online at http://www.ucl.ac.uk/taxome/jim/pap/wynne03.pdf, August 2008.

Young, A., Boyle, T. & Brown, T. (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* **11**, 413–419.

Appendix. Allele frequencies of all polymorphic loci of all populations analysed of *Aricia agestis* and *Cupido minimus*. Abbreviations of the sites as in fig. 1.

Aricia agestis

loci	alleles	1	3	4	5	6	7	10	12
Pk	1	0	0	0.038	0	0	0	0	0
	2	1.000	1.000	0.962	1.000	0.988	0.974	0.987	1.000
	3	0	0	0	0	0.013	0.026	0.013	0
G6pdh	1	0.050	0.013	0.013	0	0.053	0.013	0.013	0.075
	2	0.625	0.829	0.782	0.962	0.921	0.987	0.885	0.837
	3	0.313	0.092	0.205	0.038	0.026	0	0.077	0.063
	4	0.013	0.066	0	0	0	0	0.026	0.025
Aat2	1	0	0	0.013	0	0	0	0	0
	2	0.913	0.846	0.872	0.872	0.895	0.850	0.897	0.750
	3	0.087	0.154	0.115	0.128	0.105	0.150	0.103	0.250
Pgi	1	0.175	0.013	0.026	0.063	0.132	0.064	0.039	0.038
	2	0.200	0.218	0.115	0.162	0.092	0.179	0.066	0.218
	3	0.350	0.462	0.372	0.387	0.382	0.462	0.539	0.410
	4	0.200	0.244	0.410	0.287	0.316	0.179	0.316	0.205
	5	0.075	0.064	0.077	0.100	0.079	0.115	0.039	0.128
Hbdh	1	0.044	0.026	0.014	0.013	0.039	0	0.014	0
	2	0.338	0.171	0.135	0.066	0.184	0.030	0.129	0.081
	3	0.588	0.776	0.797	0.895	0.776	0.924	0.814	0.905
	4	0.029	0.026	0.054	0.026	0	0.045	0.043	0.014
Idh1	1	1.000	0.962	1.000	1.000	1.000	1.000	1.000	1.000
	2	0	0.038	0	0	0	0	0	0
Mdh1	1	0	0	0	0.025	0.108	0	0	0.013
	2	0.988	0.975	0.988	0.913	0.892	1.000	0.974	0.962
	3	0.013	0.025	0.013	0.063	0	0	0.026	0.025
Mdh2	1	0	0	0.013	0	0	0.013	0	0
	2	0.950	0.988	0.988	0.962	0.975	0.988	0.975	0.950
	3	0.050	0.013	0	0.038	0.025	0	0.025	0.050
Me	1	0	0	0	0	0	0	0.029	0
	2	0	0	0.026	0.051	0	0.029	0	0
	3	0.974	0.500	0.500	0.564	0.515	0.429	0.691	0.466
	4	0.026	0.500	0.474	0.385	0.485	0.543	0.279	0.534
Pep	1	0	0.050	0.066	0.122	0.050	0.125	0.054	0.175
	2	1.000	0.938	0.934	0.878	0.938	0.875	0.932	0.800
	3	0	0.013	0	0	0.013	0	0.014	0.025
Pgm	1	0.013	0.013	0.013	0	0	0.013	0.038	0.038
	2	0.087	0.224	0.141	0.063	0.013	0.050	0.077	0.038
	3	0.575	0.566	0.590	0.712	0.737	0.700	0.628	0.587
	4	0.275	0.158	0.141	0.162	0.145	0.162	0.192	0.250
	5	0.050	0.039	0.103	0.025	0.026	0.063	0.051	0.063
	6	0	0	0	0	0	0.013	0.013	0.025
	7	0	0	0.013	0.038	0.079	0	0	0
	N	40	38	39	40	38	40	39	40

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Cupido minimus

loci	alleles	2	8	9	10	11	12	13
Aat1	1	0	0.013	0	0	0.013	0	0
	2	1.000	0.962	0.974	0.961	0.925	0.981	0.950
	3	0	0.025	0.026	0.039	0.063	0.019	0.050
Pk	1	0.014	0	0	0	0	0	0
	2	0.986	1.000	1.000	0.987	0.988	1.000	1.000
	3	0	0	0	0.013	0.013	0	0
Apk	1	1.000	1.000	1.000	0.987	0.974	1.000	1.000
	2	0	0	0	0.013	0.026	0	0
6Pgdh	1	0.224	0.825	0.697	0.671	0.686	0.288	0.375
	2	0.776	0.175	0.303	0.329	0.314	0.712	0.625
G6pdh	1	0.934	0.975	0.949	0.987	0.975	0.904	0.962
	2	0.066	0.025	0.051	0.013	0.025	0.096	0.038
Gpdh	1	0	0	0	0.026	0.013	0	0
	2	0.974	1.000	1.000	0.974	0.950	1.000	0.988
	3	0.026	0	0	0	0.038	0	0.013
Pgi	1	0.013	0	0	0	0	0	0
	2	0.026	0.038	0.013	0.013	0.013	0.038	0.075
	3	0.526	0.363	0.474	0.605	0.538	0.500	0.500
	4	0.303	0.363	0.342	0.197	0.256	0.327	0.300
	5	0.132	0.237	0.132	0.132	0.141	0.096	0.112
	6	0	0	0.039	0.053	0.051	0.038	0.013
Idh1	1	0.987	1.000	1.000	1.000	0.988	1.000	1.000
	2	0.013	0	0	0	0	0	0
	3	0	0	0	0	0.013	0	0
Idh2	1	0.947	1.000	0.885	0.921	0.897	0.981	0.962
	2	0.053	0	0.115	0.079	0.103	0.019	0.038
Mdh1	1	0	0	0	0.105	0.025	0.019	0.038
	2	1.000	1.000	0.974	0.895	0.975	0.981	0.962
	3	0	0	0.026	0	0	0	0
Mdh2	1	0.987	0.988	0.987	0.934	0.974	1.000	0.988
	2	0.013	0.013	0.013	0.066	0.026	0	0.013
Me	1	0	0	0	0	0.013	0.038	0
	2	0.934	1.000	1.000	1.000	0.938	0.962	1.000
	3	0.066	0	0	0	0.050	0	0
Pep	1	0.081	0.150	0.141	0.092	0	0.038	0.063
	2	0.703	0.700	0.667	0.789	0.903	0.885	0.800
	3	0.216	0.150	0.192	0.118	0.097	0.077	0.138
Pgm	1	0.041	0	0.051	0.039	0.064	0.021	0.013
	2	0.068	0.262	0.167	0.184	0.205	0.146	0.282
	3	0.392	0.262	0.385	0.474	0.397	0.458	0.615
	4	0.324	0.450	0.308	0.184	0.282	0.292	0.051
	5	0.176	0.025	0.090	0.118	0.051	0.083	0.038
	N	40	40	40	40	40	40	40

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