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Genetic variations in space and time in *Parnassius mnemosyne* (L.) (Lepidoptera) populations in north-east Hungary: implications for conservation

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Abstract

The population structure of the clouded Apollo butterfly *Parnassius mnemosyne* was investigated by mark–release–recapture studies and by allozyme polymorphism in north-east Hungary. Large differences were observed in the estimated sizes of different populations. The results of the genetic analysis suggest that even large populations may have small effective population sizes, due to uneven sex ratio, recent bottlenecks and founder effect. The results of both the genetic and MRR studies indicated that the Bükk populations exist as a metapopulation. However, populations from different geographical regions were highly differentiated, indicating restricted gene flow between them. Loss of genetic variability was observed in a small, isolated population. Practical advice is given on how to manage woodland to maintain genetic diversity; it is concluded that many small clearings made close to existing habitat patches is superior to making fewer, larger clearings.

Keywords: Population structure; Population size; *Parnassius mnemosyne*; Metapopulation; Mark–release–recapture

1. Introduction

Conservation of biological diversity is an increasingly important task in the face of massive destruction and the fragmentation of natural habitats (Soulé, 1986). To develop an effective conservation strategy for a particular species, it is important to have information both on the genetic diversity and on the ecological characteristics of the species concerned.

The clouded Apollo butterfly *Parnassius mnemosyne* (Linnaeus, 1758) requires structured habitats; larvae feed on *Corydalis cava* and *C. solida*, at the sunny margins of humid, deciduous forests, while imagines prefer clearings for mating and feeding (Weidemann, 1986; Ebert and Rennwald, 1991). Therefore, this butterfly can only occur in habitats where both clearings and the forest with the foodplants are present.

Since the last glacial period, north-east Hungary had been continuously covered with forest until the appearance of Neolithic culture ca. 6000 years ago (Willis et al., 1997). As a consequence of human activities, extensive deforestation occurred in the valleys, resulting in the separation of the forested area of the Aggtelek karst, Bükk mountains and the hardwood gallery forests of the Tisza region (Fig. 1). The largest continuous woodland in north-east Hungary remained on the Bükk plateau until recent clear-fellings ca. 200 years ago. Concurrently, the extreme fragmentation of the gallery forests has occurred, due to the control of the Tisza river and its tributaries in the 19th century (i.e. the shortening of the river bed by cutting off its meanders), which has resulted in the isolation of the Sajólád forest (Fig. 1). Forest management not only fragmented, but also substantially altered the structure of once natural forests. Due to clear-fellings and reforestation, most forest patches consist of trees of the same age, hence they are dense. At the same time, natural clearings are replaced by clear-cut patches, which are suitable but only temporary habitats for the clouded Apollo.

P. mnemosyne is endangered in northern and central Europe (Heath, 1981; van Swaay et al., 1997) and it is included on the list of protected species by the Bern Convention. In Hungary, this species still occurs in

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strong populations. Nevertheless, several populations have already disappeared, and others are isolated and vulnerable, hence the species is protected and included in the Red Data Book of Hungary (Varga, 1990). Thus, the main goal of the present study was to investigate both the ecological characteristics and the genetic diversity of *P. mnemosyne* populations in the fragmented forests of north-east Hungary and determine an appropriate conservation strategy for the species.

Our first results on the population structure of *P. mnemosyne* were contradictory. On the basis of the allozyme data obtained in 1994 we concluded that even closely situated populations were differentiated from each other and they were strongly affected by genetic drift. The genetic composition of these populations could not fit into a hierarchical structure (Meglécz et al., 1997a). On the contrary, the results from 1995 indicated clear differentiation among regions and a rather homogenous gene pool within the Bükk mountains. Obvious differences in population size were observed in the investigated area, so we distinguished large and small populations. Analysing the data from the 2 years, for these two groups separately, we found that large populations were genetically more stable and showed geographic structure (i.e. differentiation between regions, and no significant differences within regions or between generations), while small populations were strongly affected by genetic drift, which resulted in differentiation within a region and between generations (Meglécz et al., 1997b). Since the conclusions drawn from samples collected in two consecutive years were substantially different, it was necessary to follow the same populations for further generations.

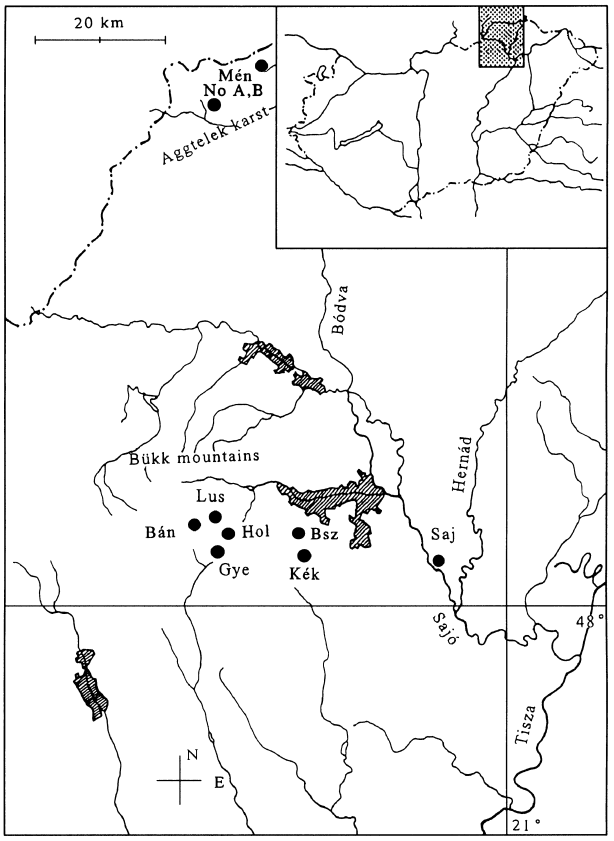

Fig. 1. Sampling localities at ten *Parnassius mnemosyne* populations in NE Hungary. Aggtelek karst: Nagyoldal-A (NoA), Nagyoldal-B (NoB), Ménes valley (Mén); Bükk mountains: Lusta valley (Lus), Bányahegy (Bán), Gyertyán valley (Gye), Hollóstetö (Hol), Bükkszentlászló (Bsz), Kékmezö (Kék); Sajólád isolate (Saj). Shaded areas are towns.

2. Materials and methods

2.1. Samples

Ten populations were sampled from three different regions (Fig. 1): the Aggtelek karst (Nagyoldal-A, Nagyoldal-B, Ménes valley), the Bükk mountains (Lusta valley, Bányahegy, Gyertyán valley, Hollóstetö, Bükkszentlászló, Kékmezö), and Sajólád isolate. Samples were collected at each site in at least 3 years between 1994 and 1997 (Table 1).

2.2. Electrophoretic studies

Imagines were collected in late May, and stored at −20°C until electrophoresis. Eight loci were examined in all samples: glutamate-oxalacetate transaminase (*Got*, EC 2.6.1.1), α-glycerophosphate dehydrogenase (α-*Gpdh*, EC 1.1.1.8), hexokinase (*Hk*, EC 2.7.1.1), isocitrate dehydrogenase (*Idh*, EC 1.1.1.42), malate dehydrogenase (*Mdh*, EC 1.1.1.37), phosphoglucose isomerase (*Pgi*, EC 5.3.1.9), phosphoglucomutase (*Pgm*, EC 2.7.5.1) and superoxide dismutase (*Sod*, EC 1.15.1.1) and three of them were polymorphic (*Hk*, *Pgi*, *Pgm*). Electrophoresis was carried out on horizontal starch gel slabs according to Meglécz et al. (1997a).

2.3. Mark–release–recapture studies

Mark–release–recapture (MRR) studies were carried out at two sites, to investigate dispersion between habitat patches and estimate population sizes and sex ratio.

The first site was in Sajólád forest (Fig.1; Saj), which is a hardwood gallery forest of the Sajó river, near the Tisza valley on the northern margin of the Great Hungarian Plain (ca. 90 m altitude). Here the *P. mnemosyne* population was small and completely isolated. Most of the imagines were found within a small clearing (ca. 150×400 m), while some individuals were flying in a narrow (15–20 m) glade about 600 m away from the clearing separated by a dense, mixed hardwood gallery forest.

The second site was at Bányahegy (Fig.1; Bán), which is situated on the Bükk plateau ca. 800 m above sea level, in the mountain beech *Fagus sylvatica* zone, where

P. mnemosyne was very abundant. The habitat at Bányahegy was rather different from the one at Sajólád. There were several small clearings, and the largest ones were slightly larger than 100×100 m. They were often connected to each other and the vegetation separating them was either a young beech scrub or old, sparse, beech forest. Four of these clearings were chosen for MRR study each year: patch A, B, U and G in 1995 and patch A, B, U and H in 1996 (Fig. 2). Patch G was dropped in 1996, because very few individuals had been captured there in 1995; patch H was added instead, which was discovered during the 1996 field season.

Butterflies were marked individually with a permanent marker pen on the underside of the hindwing, and then released immediately after marking. Both sites were studied in 2 years on the following capture days: Bányahegy, 1995: May 23, 26, 29, 31, June 6, 9; Bányahegy 1996: May daily 20–27, 30, 31, June daily 1–4, 6, 8, 11; Sajólád 1995: May 10, 12, 16, 19, 22, 24, 25, 26, 28, 30, 31, June 1; Sajólád 1996: May 13, 15, 17, 19. In 1996, weather permitting, all the habitat patches were visited within a day at Bányahegy. In bad weather conditions, however, only one or two patches were visited, with the emphasis on patch U, in order to ensure a good data set for this patch. In all other cases, habitat patches of the given site were surveyed with the same capture effort within any day. In Bányahegy totals of 598 and 1581 individuals were marked in 1995 and 1996, respectively, and in Sajólád 144 and 221 individuals.

Table 1
Allele frequencies in three polymorphic loci for 10 populations of *Parnassius mnemosyne*

	Hk *n*[b]	1	2	3	*Pgm* *n*	1	2	3	4	5	6	*Pgi* *n*	1	3	5
NoA94[a]	57	0.272	0.684	0.044	53	0.009	0.557	0.330	0.104	–	–	59	0.110	0.881	0.008
NoA95	45	0.311	0.667	0.022	45	0.033	0.500	0.356	0.100	–	0.011	46	0.033	0.967	–
NoA96	37	0.351	0.649	–	36	0.069	0.486	0.319	0.111	–	0.014	31	0.065	0.887	0.048
NoA97	34	0.279	0.632	0.088	34	0.074	0.441	0.368	0.103	–	0.015	36	0.056	0.944	–
NoB95	39	0.308	0.641	0.051	40	0.038	0.250	0.500	0.200	–	0.013	40	0.038	0.950	0.013
NoB96	39	0.295	0.654	0.051	36	0.083	0.403	0.361	0.153	–	–	39	0.077	0.910	0.013
NoB97	37	0.284	0.635	0.081	37	0.068	0.432	0.446	0.054	–	–	39	0.051	0.923	0.026
Mén94	16	0.813	0.188	–	17	0.029	0.500	0.265	0.088	0.118	–	20	0.050	0.950	–
Mén95	14	0.500	0.500	–	14	–	0.250	0.393	0.357	–	–	14	0.036	0.893	0.071
Mén96	12	0.667	0.333	–	11	0.045	0.545	0.182	0.182	–	0.045	11	0.091	0.909	–
Mén97	12	0.625	0.292	0.083	12	0.125	0.542	0.208	0.083	–	0.042	10	0.050	0.950	–
Lus94	53	0.698	0.302	–	50	0.120	0.680	0.150	0.050	–	–	33	0.333	0.667	–
Lus95	36	0.597	0.403	–	34	0.118	0.706	0.118	0.044	0.015	–	36	0.167	0.833	–
Lus96	51	0.627	0.373	–	51	0.078	0.765	0.137	0.020	–	–	51	0.196	0.784	0.020
Lus97	43	0.512	0.488	–	42	0.119	0.560	0.250	0.048	–	0.024	43	0.163	0.756	0.081
Báh94	43	0.663	0.314	0.023	49	0.082	0.786	0.133	–	–	–	33	0.242	0.742	0.015
Báh95	81	0.654	0.346	–	87	0.063	0.678	0.195	0.052	0.011	–	87	0.161	0.822	0.017
Báh96	92	0.549	0.451	–	92	0.087	0.712	0.179	0.016	0.005	–	91	0.181	0.786	0.033
Báh97	43	0.547	0.453	–	43	0.070	0.756	0.163	0.012	–	–	42	0.167	0.810	0.024
Gye94	48	0.469	0.521	0.010	49	0.031	0.796	0.082	0.092	–	–	48	0.125	0.750	0.125
Gye95	27	0.519	0.481	–	29	0.069	0.603	0.207	0.121	–	–	29	0.155	0.741	0.103
Gye96	44	0.602	0.398	–	44	0.057	0.648	0.170	0.125	–	–	44	0.205	0.705	0.091
Gye96	39	0.551	0.449	–	39	0.026	0.679	0.167	0.128	–	–	39	0.141	0.769	0.090
Hol94	44	0.682	0.318	–	40	0.063	0.613	0.188	0.138	–	–	44	0.159	0.727	0.114
Hol95	29	0.672	0.310	0.017	28	0.018	0.643	0.232	0.071	0.036	–	29	0.241	0.707	0.052
Hol96	31	0.468	0.516	0.016	31	0.048	0.548	0.226	0.161	0.016	–	31	0.177	0.758	0.065
Bsz94	24	0.563	0.438	–	30	0.033	0.683	0.283	–	–	–	31	0.145	0.645	0.210
Bsz95	22	0.500	0.500	–	24	0.042	0.688	0.250	0.021	–	–	24	0.208	0.750	0.042
Bsz97	26	0.615	0.385	–	26	–	0.596	0.346	0.058	–	–	26	0.269	0.692	0.038
Kék95	23	0.652	0.348	–	23	0.130	0.609	0.152	0.065	0.043	–	23	0.239	0.674	0.087
Kék96	21	0.571	0.429	–	21	0.048	0.690	0.214	0.048	–	–	25	0.260	0.680	0.060
Kék97	33	0.591	0.409	–	32	0.078	0.656	0.156	0.109	–	–	33	0.076	0.894	0.030
Saj94	38	0.566	0.434	–	42	0.167	0.548	0.286	–	–	–	45	0.178	0.822	–
Saj95	22	0.432	0.568	–	18	0.056	0.583	0.333	–	0.028	–	22	0.295	0.705	–
Saj96	36	0.472	0.528	–	40	0.125	0.625	0.237	–	0.013	–	41	0.195	0.805	–
Saj97	42	0.476	0.524	–	41	0.207	0.439	0.354	–	–	–	42	0.095	0.905	–

[a] Populations: Nagyoldal-A (NoA), Nagyoldal-B (NoB), Ménes valley (Mén), Lusta valley (Lus), Bányahegy (Bán), Gyertyán valley (Gye), Hollóstetö (Hol), Bükkszentlászló (Bsz), Kékmezö (Kék) Sajólád (Saj).

[b] *n*: Sample sizes.

2.4. Data analyses

2.4.1. Allozyme data

Deviation from the Hardy–Weinberg equilibrium, linkage disequilibrium and genetic heterogeneity among the entire set, or for a group of samples were analysed by Fisher's exact test, using the Genepop package (Raymond and Rousset, 1995). The same package was used to carry out Slatkin and Barton's (1989) isolation-by-distance test. Two types of *F*-statistics were carried out: Weir and Cockerham's (1984) θ values were calculated in the non-hierarchical analyses (corresponding to F_{ST} in Wright, 1978) using the program Fstat, version 1.2 (Goudet, 1995). This program tests the significance of θ by using permutations. In hierarchical analyses, Wright's (1978) estimators were calculated by using the program Biosys-1, Release 1.7 (Swofford and Selander, 1981). Here the total between population variation (F_{PT}) was divided into differentiation within region (F_{PR}) and between regions (F_{RT}) for each year separately.

Effective population size of the Sajólád isolate was calculated from the temporal changes of allele frequencies. Following Pollak's (1983) suggestion, we used Nei and Tajima's (1981) estimator ($\hat{F}_c$) for loci with two alleles, but Pollak's estimator ($\hat{F}_k$) for loci with three or more alleles to calculate the temporal variance in allele frequencies. The mean of $\hat{F}$ values over loci weighted by the number of alleles were used to calculate the effective population size according to Eq. 18 in Nei and Tajima (1981). The 95% confidence limit of the estimated effective population size was calculated according to Waples (1989).

2.4.2. MRR data

Population estimates were made using general Jolly–Seber models (Schwarz and Arnason, 1996), with the POPAN-4 and POPAN-5 programs (Arnason and Schwarz, 1995; Arnason et al., 1998). Both survival and probability of capture were allowed to vary with time and sex of the individuals for each of the four locality×year combinations. Days were often grouped into two or occasionally three day sessions, in order to increase the probability of capture at each session, and hence to reduce the confidence interval of daily population size and birth estimates. Capture probabilities at the first and last sessions of each site-year were estimated by setting the probabilities of capture equal for the first two and the last two sessions, respectively. Total population sizes of imagines were estimated by maximum likelihood methods based on both birth and survival rates (Schwarz and Arnason, 1996), using the POPAN-5 UFIT procedure. In all cases, gross population totals were retained in order to include individuals which emerged during an interval between sessions, but did not survive until the next trapping session, assuming that these have the same survival rate as the captured individuals. The capture–recapture methods used here estimate total butterfly emergence in a way analogous to fish biologists estimating total escapement of migrating fish passing a given stretch of river (Schwarz et al., 1993).

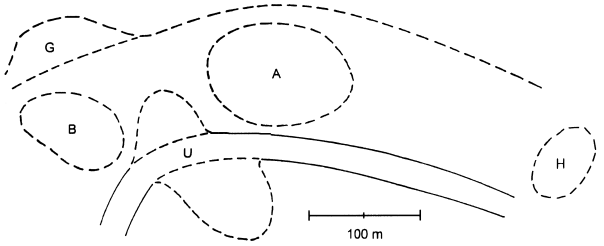

Fig. 2. Habitat patches investigated by MRR at Bányahegy. Patches A, B, U and G were studied in 1995, and A, B, U and H in 1996.

In the Bányahegy population, the number of capture days was lower in 1995 (6 days) than in 1996 (17 days). As the total estimate was only valid for the period of capture, the estimated population size was far lower in 1995 than in 1996. A truncation of the 1996 trapping session to 6 days spaced as the 1995 trapping session, however, gave an estimate of the same order of magnitude as the 1995 estimate. To obtain an estimate of the total number of males during the whole 1995 flight season in the Bányahegy population, the 1995 estimate was multiplied by the ratio of the estimate from the total 1996 data (2264) to the estimate from the truncated 1996 data (1090). This approach does not hold for Sajólád, as in 1995 the site was visited during the whole flight season, which is much shorter there then at Bányahegy.

Overall sex ratios (females/males) were estimated for Bányahegy 1996 and Sajólád 1996 populations. Their 95% confidence intervals were calculated using the estimated variances (V_m and V_f) of the total estimated populations of males (M) and females (F). The variance of the ratio was estimated as

$$V_r = \left(\frac{F}{M}\right)^2 \left(\frac{V_m}{M^2} + \frac{V_f}{F^2} - \frac{2\mathrm{cov}(M,F)}{MF}\right),$$

where the covariance term is nil, as the estimates of male and female populations were obtained without any between-group constraint.

3. Results

3.1. Allozyme data

Allele frequencies are shown in Table 1. The average number of alleles in the Sajólád samples were significantly lower than in all other samples (Mann–Whitney's U test, $p < 0.001$). Allele 5 at *Pgi* and allele 4 at

Table 2
Differentiation among different sets of populations revealed by θ values (A) and exact test for population differentiation (B)[a]

		All populations				Bükk and Sajólád populations				Bükk populations			
		1994	1995	1996	1997	1994	1995	1996	1997	1994	1995	1996	1997
A	*Hk*	0.093**	0.065**	0.038**	0.036*	0.021*	0.014	0.003	−0.005	0.027*	0.003	0.000	−0.010
	Pgm	0.040**	0.068**	0.032**	0.042**	0.034**	−0.001	0.010	0.039**	0.023**	−0.004	0.011	0.017*
	Pgi	0.044**	0.051**	0.015*	0.035**	0.025**	0.009	−0.001	0.021**	0.021**	0.007	−0.001	0.013
	All loci	0.061**	0.063**	0.030**	0.038**	0.027**	0.007	0.004	0.019**	0.024**	0.002	0.003	0.006
B	*Hk*	***	***	***	***	**	*	NS	NS	**	NS	NS	NS
	Pgm	***	***	***	***	***	NS	***	***	***	NS	**	***
	Pgi	***	***	**	***	***	*	NS	*	***	*	NS	NS
	All loci	***	***	***	***	***	**	***	***	***	NS	**	**

[a] * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Pgm were missing from all Sajólád samples, while they were present in almost all Bükk samples. Allele 3 at *Hk* and allele 6 at *Pgm* were also missing at Sajólád, but these alleles were also rare in the Bükk samples. Significant linkage disequlibrium was detected in only three cases out of 108 comparisons (Lus-1996, *Hk*-*Pgm*, $p < 0.01$; Gye-1996, *Hk*-*Pgm*, $p < 0.05$; Saj-1997, *Hk*-*Pgi*, $p < 0.01$), Fisher's exact test over populations did not indicate disequilibrium in any of the combinations of the loci (*Hk*-*Pgm*, $p = 0.160$; *Hk*-*Pgi*, $p = 0.702$; *Pgm*-*Pgi*, $p = 0.781$).

Significant deficiency of heterozygotes (compared to the Hardy–Weinberg expectations) was observed in 13 cases out of the 108 comparisons; in nine cases at *Hk* (NoB95, $p < 0.01$; Mén97, $p < 0.05$; Lus94, $p < 0.01$; Bán94, $p < 0.001$; Gye94, $p < 0.001$; Gye95, $p < 0.01$; Gye96, $p < 0.01$; Gye97, $p < 0.001$; Hol94, $p < 0.05$), in three samples at *Pgm* (NoA97, $p < 0.05$; Mén94, $p < 0.05$; Bán96, $p < 0.05$) and only in one sample at *Pgi* (Bsz94, $p < 0.05$). Fisher's exact test over loci indicated an overall deficit of heterozygotes at the *Hk* ($p < 0.001$) and at the *Pgm* loci ($p < 0.001$), while *Pgi* proved to be in Hardy–Weinberg equilibrium ($p = 0.210$).

When all samples were considered, both exact test for population differentiation and θ values indicated strong differentiation in each year. The level of differentiation became smaller, when the Aggtelek samples were removed (i.e. among Bükk and Sajólád samples), and decreased even further, when only the Bükk populations were considered (Table 2). Isolation by distance was observed when all populations were included in the analyses ($p < 0.05$), in every year separately and in the pooled data set as well. This phenomenon, however, was not detected when only the Bükk populations were analysed. Hierarchical F-statistics indicated that the level of differentiation varied over time, and both the within-region and the between-region components of the total variability among populations are important (Table 3). The total between-population variability (F_{PT}) was higher in 1994 and 1995 than in the other two years. Variability between and within regions also varied. The two extremes were 1994, when most of the variability was found within a region and 1995 when strong differentiation was observed between regions.

Effective population sizes of the Sajólád population were estimated from all possible sample pairs. The estimates varied between pairs and had wide confidence

Table 3
Results of hierarchical F-statistics

	1994	1995	1996	1997	Pooled data set
F_{PR}[a]	0.058	0.014	0.026	0.024	0.022
F_{RT}[b]	0.006	0.062	0.013	0.011	0.020
F_{PT}[c]	0.052	0.076	0.039	0.034	0.041

[a] F_{PR} measure of variation among populations within regions.
[b] F_{RT} measure of variation among regions.
[c] F_{PT} measure of total variation among populations.

Table 4
Estimated temporal variance of allele frequencies ($\bar{F}$) and effective population sizes (N_e) of the Sajólád population

Time	No. of alleles	N_e	$\bar{F}$
1969–1972[a]	16	8 (3–19)[b]	0.263 (0.144–0.630)
1969–1995[a]	16	∞ (342–∞)	0.037 (0.020–0.089)
1972–1995[a]	17	66 (23–171)	0.242 (0.134–0.561)
1994–1995	8	18 (2–∞)	0.066 (0.029–0.273)
1994–1996	8	∞ (19–∞)	0.019 (0.008–0.079)
1994–1997	7	125 (10–∞)	0.036 (0.016–0.175)
1995–1996	8	∞ (6–∞)	0.031 (0.014–0.128)
1995–1997	8	15 (3–114)	0.107 (0.047–0.443)
1996–1997	8	21 (3–∞)	0.049 (0.021–0.203)

[a] Data from Meglécz et al. (1998) based on three microsatellite loci.
[b] 95% confidence intervals are in parentheses.

intervals (Table 4). Differentiation among generations of the same population (i.e. variation over time) was only found in Lusta and Ménes valleys (Table 5).

3.2. MRR data

More than a 10-fold difference was observed between the number of males in the Bányahegy and the Sajólád populations. The population size at Sajólád increased from 1995 to 1996, while it remained more constant in Bányahegy (Table 6). Since the recapture probabilities of females were often low, the number of females could not be estimated at any of the sites in 1995. In 1996, overall sex ratios (F/M) were 0.68 (95%CI: 0.41, 0.95) and 0.62 (95%CI: 0.47, 0.77) in Sajólád and Bányahegy, respectively.

The dispersal rate was estimated by dividing the number of individuals which were recaptured in at least two different patches, by the total number of individuals recaptured (Table 7). The rate of dispersal was not significantly different between years at either of the two sites. (Bán-95: 25.3%, Bán-96: 24.5%; Saj-95:8.1%; Saj-96:17.2%) Considering the 2 years together, the overall dispersal rate was significantly greater in Bányahegy than in Sajólád ($p < 0.001$). Analysing the years separately, this rate was significantly greater at Bányahegy than at Sajólád in 1995 ($p < 0.001$), but not in 1996 ($p = 0.125$). Males moved more frequently across habitat patches than females at Bányahegy in 1996 ($p < 0.001$). Although this comparison was not significant at Sajólád and at Bányahegy in 1995, in the later three data sets the number of recaptured females were low. Males showed a significantly higher dispersal rate than females after pooling all four data sets ($p < 0.001$).

Table 5
Probability values of exact test for differentiation between generations of the same populations

	p
NoA 94-95-96-97	0.125
NoB 95-96-97	0.238
Mén 94-95-96-97	0.014*
Lus 94-95-96-97	0.002**
Bán 94-95-96-97	0.088
Gye 94-95-96-97	0.544
Hol 94-95-96	0.125
Bsz 94-95-97	0.062
Kék 95-96-97	0.088
Saj 94-95-96-97	0.062

Table 6
Total number of individuals at the two sites in 1995 and 1996 estimated from MRR studies

		Sajólád	Bányahegy
1995	Males	124	2143
	Females	60??	??
1996	Males	233 (186, 280)[a]	2264 (2019,2509)
	Females	158 (103, 212)	1401 (1103,1699)
Sex ratio F/M		0.68 (0.41,0.95)	0.62 (0.47,0.77)

[a] 95% confidence intervals are in parentheses, where available.

4. Discussion

4.1. Deficit of heterozygotes

An overall deficit of heterozygotes was found in the populations, which often resulted in significant Hardy–Weinberg disequilibrium. This phenomenon was not restricted to allozyme markers, but was also observed at microsatellite loci (Meglécz et al., 1998). In theory, there are five possible causes for the deficit of heterozygotes: (i) underdominant selection, (ii) presence of null alleles, (iii) population subdivision, (iv) assortative mating or (v) inbreeding (Endler, 1986). In Meglécz et al. (1998) we concluded that, although none of the alternatives can be completely excluded, inbreeding and population subdivision were more likely to have caused the observed pattern.

Since collection sites of the butterflies usually were well defined clearings, population subdivision within these clearings could only occur if they were feeding and basking sites for more than one subpopulation. In addition, the assumption of population subdivision requires that butterflies from a given subpopulation do not, or rarely, interbreed with individuals coming from a different subpopulation. This assumption does not hold for two reasons. (i) Several copula were observed on the clearings. (ii) Females lay their eggs in the forest near the clearings. Wherever they find an appropriate place they land on the ground or on the undergrowth, lay one or a few eggs, and then fly a few hundred metres until they find a new place to lay further eggs. This process is repeated several times until all eggs are deposited (pers. observation). Thus the eggs of a single

Table 7
Dispersal between habitat patches

		Females	Males	Total
Bányahegy-95	Stay[a]	9	103	112
	Move[b]	4	34	38
Bányahegy-96	Stay	96	286	382
	Move	10	114	124
Sajólád-95	Stay	19	60	79
	Move	0	7	7
Sajólád-96	Stay	13	40	53
	Move	3	8	11

[a] Stay: Number of individuals recaptured only in the patch of their initial marking.

[b] Move: Number of individuals found in at least two different patches (dispersing individuals).

female are dispersed over a large area near the clearing.

Inbreeding occurs in small populations. Although it seems unlikely that large populations like the one at Bányahegy could be affected by inbreeding, yet this is not impossible, since the census population size and the genetic effective population size can differ greatly. Effective population size is reduced by uneven sex ratios, population bottlenecks, differences in reproductive success of individuals and founder effects. Our results suggested that the sex ratio is uneven, and great changes in population size can occur (Sajólád). Many studies have reported that one single year with unfavourable conditions could cause a serious bottleneck in the populations (Warren, 1987a; Pollard and Yates, 1993 and references therein). Our field experiences also indicated strong fluctuation in population size due to weather conditions (unpublished data). Most of the investigated populations were found on clear cut areas (for example at Bányahegy, Lusta valley, Hollóstetö), which have already been colonised by wild flowers, but have not yet been re-forested. These sites are temporary since they disappear once the saplings are high enough to shade out the undergrowth. This implies that extinction of certain populations and founding of new populations are frequent, if migration rate is low among populations.

The results of exact test for population differentiation indicate (Table 2), that there are significant genetic differences between Bükk populations; this shows that the migration rate is not high enough to counterbalance the genetic differentiation among populations. Our finding on the dispersal rate can also indirectly support this hypothesis. Although the dispersal rate was estimated only for short distances (a few hundred metres), even closely situated habitat patches with open (sunny) access to each other did not freely exchange individuals (Bányahegy). The same phenomenon was also observed in *P. mnemosyne* populations in southern France (Napolitano et al., 1988) and the authors concluded that the populations of this species are rather closed. We also found that the dispersal rate was significantly higher in Bányahegy, where habitat patches are connected than in Sajólád, where the patches were separated by forest. Furthermore, in a MRR study conducted in Norway, in four *P. mnemosyne* populations, no individuals were observed to disperse between habitat patches separated by only 2 km (Aagaard and Hanssen, 1992). Dispersal rate of the females was also found to be lower than that of the males. This results in a very biased sex ratio in the small founder population. As a consequence, even if the newly founded population reaches a large population size within a few generations, its effective population size will remain small.

The patchy habitat structure, and the relatively restricted dispersal between local populations together with the temporary character of many habitat patches suggest a metapopulation structure within the Bükk mountains. Hedrick and Gilpin (1997) have investigated the influence of several factors on the effective size of metapopulations. According to the results of their simulations, the carrying capacity (and thus the census population size) of a local population has relatively small effect on its effective size. Their results support our hypothesis on small effective size of local *P. mnemosyne* populations.

4.2. Geographic differentiation

Hierarchical F-statistics indicated both between- and within-region differentiation (Table 3). A considerable part of the total genetic variability among populations was allocated between regions in all data sets except in 1994. The highly significant differentiation detected among all samples by both exact test for population differentiation and θ values (Table 2) decreased greatly when removing the Aggtelek samples (i.e. among the Bükk and Sajólád samples), and a further decrease was observed after excluding the Sajólád samples (i.e. among the Bükk samples only). This indicated strong differentiation between the regions. Similar conclusions can be drawn from the results of the isolation-by-distance test. When including all populations, isolation-by-distance was detected in every year owing to the fact that the greatest genetic differences were found between Bükk and Aggtelek populations, which correlated with the great geographical distance between them. On the other hand, no isolation by distance was detected among Bükk populations.

The Sajólád population is separated by only about 15 km of unsuitable habitat from the nearest Bükk populations, but its genetic structure is clearly different from that of the Bükk region. Out of the 12 alleles present in the Bükk populations four were missing in all Sajólád samples. The lack of allele 4 at *Pgm* and allele 5 at *Pgi* is striking, as these alleles are present in almost all Bükk samples. Since the gallery forests of the Tisza river and the forests of the Bükk mountains have been connected up until about 200 years ago, it is likely that these alleles were present in the Sajólád population, but have been lost. As it is a small population, the most likely explanation for the loss of genetic variability is genetic drift. Although selection could also be responsible for the genetic differences, it is unlikely that all three loci are selected for different alleles in the Bükk and in the Sajólád populations. Since no significant linkage disequilibrium was observed between any pairs of loci, the combined effect of selection and genetic hitch-hiking is also less probable. In contrast, the loss of genetic variability in small isolated populations is a frequently observed phenomenon (Bijlsma et al., 1991; Brakefield, 1992) and it has already been shown in *P. mnemosyne* populations in southern France (Descimon

and Napolitano, 1993a, 1993b). We have shown in Meglécz et al. (1998), that this population has experienced a recent bottleneck. Estimation of effective population size from temporal allele frequency changes of three microsatellite loci were not reliable. In the present study, we again had three polymorphic loci but the four sampling dates allowed six estimates (Table 4). The results are similar to the one obtained in Meglécz et al. (1998). Most of the estimated effective population sizes are small, but the upper confidence limits are often infinite. In three cases, the effective population size estimate was infinite, which was due to large sampling errors compared to the variance of allele frequencies. Differences between the estimates obtained from different pairs of samples may be the consequence of small sample sizes and the small number of loci. Nevertheless, these results also indicated that the effective population size should be small at Sajólád.

4.3. Genetic differentiation among generations

Our earlier results suggested that the genetic stability of populations is determined by their sizes and geographical locations (Meglécz et al., 1997b, 1998). Large populations situated in the central region (the Bükk plateau) of the area (Bányahegy, Lusta valley), are expected to be stable. Variation between generations in these populations is therefore supposed to be lower than in the small populations of the mountain foreground (Bükkszentlászló, Kékmezö). The results of the exact test for differentiation among generations of the same populations (Table 5) did not fully support this assumption. Significant differentiation was only found in two populations, in Ménes valley and in Lusta valley. At the same time p values were also low for the Bányahegy, Bükkszentlászló, Kékmezö, and Sajólád populations. Thus, isolated or peripheral small populations (Sajólád, Kékmezö, Bükkszentlászló, Ménes valley) show little genetic stability among generations. Nevertheless, the two largest populations situated on the Bükk plateau (Bányahegy, Lusta valley) were not stable either. This finding is in accordance with the hypothesis discussed above, that even the large populations may have small effective population sizes. The possibility of differentiation among generations is frequently overlooked, and conclusions on the genetic structure of the populations are usually drawn from samples of a single generation. Our study clearly indicated that at least in species with small populations or unstable habitats a repeated sampling is recommended.

5. Conservation implications

Hedrick and Gilpin (1997) pointed out that genetic diversity may be lost much faster in a metapopulation structure than predicted from either census population size or from traditional estimates of effective population size. Their work also gives guidelines for conservation biologists. Effective population size and the level of genetic variability can remain higher in a metapopulation with low rate of extinction, high number of founder individuals and large number of habitat patches.

This can be attained for *P. mnemosyne* populations with well planned forest management. As small clearcut spots in forested areas are generally suitable but temporary habitats for *P. mnemosyne*, small patches should be cleared instead of deforesting large areas. Furthermore, to maintain a population continuously, new clearings should be generated in the vicinity of the existing population concomitantly with the succession rate of the old habitat patch. If the patch populated by *P. mnemosyne* is planted with fast growing spruce *Picea abies* saplings, a new habitat patch should be created close to the old patch within 3 years. If only naturally regenerating beech saplings are present, the habitat patch remains suitable for this butterfly for a longer period (7–10 years). Similar management plans have proven to be efficient in maintaining healthy populations of *Mellicta athalia* in Britain (Warren, 1987), which are mostly found in short-lived types of habitat.

Since dispersal rate was high between habitat patches situated only a few hundred metres apart from each other, a significant part of the population of the declining habitat patch could shift into the newly formed patch. This could maintain the local populations continuously, with only a small (if any) decline in the population size. Several local populations should be 'managed' in this manner, in order to maintain a large effective size for the total metapopulation.

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