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SHORT COMMUNICATION

Spatial and temporal population genetic structure of the butterfly *Aglais urticae* L. (Lepidoptera, Nymphalidae)

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Abstract

The genetic diversity and the temporal and spatial genetic population structure of the butterfly *Aglais urticae*, a highly mobile species, were studied by allozyme electrophoresis. High levels of allozyme diversity were found. Most of the total genetic diversity occurred at the within-population scale rather than at the between-population scale. This variation could not be accounted for by Wright's model of 'isolation by distance'. No significant temporal variation was observed for those populations that were sampled in different years. A process combining high movement rate between neighbouring patches, long-distance migration and rare extinction/recolonization is suggested to explain the observed genetic structure. This hypothesis is favoured over an island model of population structure because migration in *A. urticae* is uniform neither with distance nor with time.

Keywords: *Aglais urticae* (small tortoiseshell), allozymes, extinction/colonization, isolation by distance, population genetic structure

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Introduction

Genetic studies on butterflies have largely focused on species with mainly closed populations (e.g. Johannesen *et al*. 1996). Species with high movement rate have been studied mainly from the point of view of population differentiation (e.g. Porter & Geiger 1995). However, some species show a high level of movement, often described as migratory, but the relative importance of temporal and spatial differentiation has rarely been assessed in such species.

Aglais urticae is a widespread species in Europe (Tolman & Lewington 1997) and its migratory capacity has been known for some time (Roer 1968). The local population dynamics of this species include high fluctuations and migration events. As a result, the relative importance of spatial and temporal differentiation of populations is also dependent on local occurrences of migration, extinction and colonization.

The aim of the present study is to investigate the relative contribution of time and space in the population genetics structure of *A. urticae*.

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Materials and methods

The species

Aglais urticae displays from one to three generations per year, depending largely on the climatic conditions. Although rather isolated at the start of the summer, individuals of this species can be seen at high densities in August and September, feeding on nectar-rich plants such as buddleias (*Buddleja* sp.), before entering adult diapause. *A. urticae* is not a specialist butterfly. Most types of habitat are suitable as long as nectar-rich flowers and patches of *Urtica dioica*, the caterpillar's host plant, are present. Its large geographical distribution and its high mobility are important factors determining the population structure of this species. On one hand, given the high rate of population fluctuations, one would expect a high rate of genetic variation in this species (Pollard & Hall 1989). On the other hand, the high movement rate and migratory habit would tend to homogenize populations over large areas.

Collection sites

Butterflies were captured from nine sites in Belgium, The

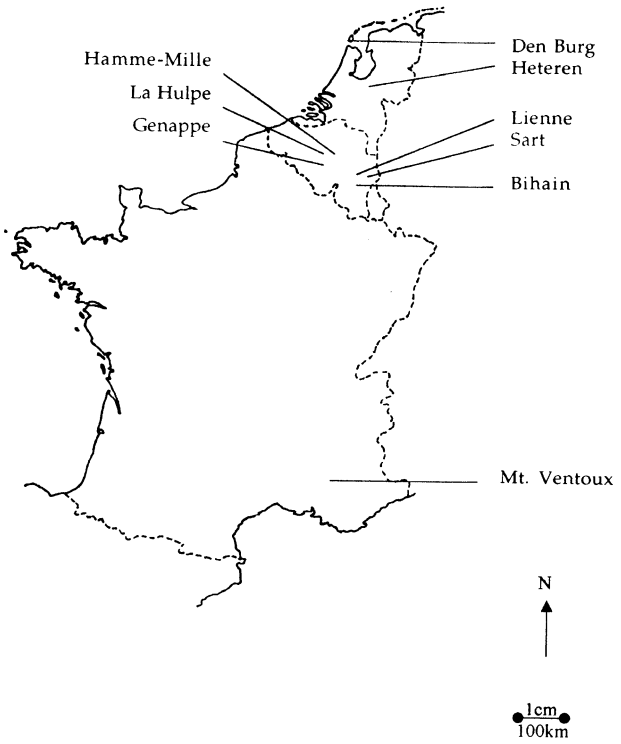

Fig. 1 Geographic locations of the nine *Aglais urticae* populations sampled in The Netherlands, Belgium and France.

Netherlands and France (Fig. 1). Sampling was designed to estimate the relative importance of local and regional variation. Populations were grouped a priori into three geographical regions: (1) the Brabant region (Belgium), where three populations were sampled; (2) the Ardennes region (Belgium), where three populations were sampled; and (3) The Netherlands where two populations were sampled. The single site sampled in the south of France will be considered as an outgroup in this study. At the latter site, individuals were collected at the caterpillar stage (seven different egg batches were sampled) and reared in the laboratory. Within each region, distances between sampled populations ranged from a few to 20 km. The distance between regions varied from 100 km to more than 1000 km.

Electrophoresis

Butterflies were stored at −80 °C in the laboratory within 24 h of their capture. At the time of analysis, the individuals were defrozen and the thorax and half of the abdomen were used for allozyme electrophoresis. These were homogenized, by squashing, in 800 µL of extraction buffer (pH 7.1, (w/v) 15% sucrose, 50 mM Tris-HCL pH 7.1, 0.5% (v/v) Triton X-100, and a drop of Bromophenol blue as runner marker) and were centrifuged at 2178 *g* for 3 min (Wynne *et al*. 1992).

Analysis was done by cellulose acetate electrophoresis following Richardson *et al*. (1986). Ten enzyme systems proved to be polymorphic. Unfortunately, only four enzyme systems representing five loci were interpretable: (i) glucose-phosphate isomerase (GPI, E.C. 5.3.1.9); (ii) β-hydroxybutyrate dehydrogenase (HBDH, E.C. 1.1.1.30); (iii) glutamate-oxaloacetate transaminase (GOT, E.C. 2.6.1.1); (iv) isocitrate dehydrogenase (IDH1 & IDH2, E.C. 1.1.1.42).

Data analysis

All of the loci tested showed banding patterns consistent with known quaternary structure (Richardson *et al*. 1986). Standards (individuals that were run previously) were included in each electrophoretis run.

Three coefficients measuring genetic variation were computed using GENSURV (Vekemans & Lefèbvre 1997): (i) the percentage of polymorphic loci (*P*) using the 0.95 criterion (a locus is considered polymorphic if the most common allele has a frequency of less than 95% in all of the populations analysed); (ii) the mean number of alleles per locus (A); and (iii) the expected heterozygosity (H_E) under Hardy–Weinberg equilibrium (Nei 1978). These three coefficients were estimated for each of the populations sampled. The mean and standard deviation of the previously mentioned coefficients were then calculated over all populations sampled.

Compliance to Hardy–Weinberg equilibrium was tested for each locus in each one of the populations sampled using GENEPOP (Raymond & Rousset 1995). This software was also used to test genetic differentiation between populations. No significant genetic differentiation was ever found between males and females, nor was any heterosomal inheritance detected. Males and females were consequently pooled for further analyses.

Partitioning of variation among and within populations was examined by means of gene diversity analysis (using GENSURV by Vekemans & Lefèbvre 1997) including the total genetic diversity (H_T), the genetic diversity among populations (D_{ST}), and the proportion of the total genetic diversity due to population differentiation ($G_{ST} = D_{ST}/H_T$). Confidence intervals of mean statistics were obtained by bootstrapping over loci using GENSURV (Vekemans & Lefèbvre 1997).

In order to test for isolation by distance, correlation between the matrix of geographical distances and the matrix of genetic distances (computed after Reynolds *et al*. 1983) was tested using a Mantel test with 1000 permutations by GENSURV (Vekemans & Lefèbvre 1997). Several genetic distances were also computed between all pairs of populations. These included Nei's (1978) unbiased genetic distance, the Cavalli-Sforza & Edwards (1967) arc distance and the Rogers (1972) genetic distance (Rogers 1986). The various genetic distance matrices

were used in an UPGMA cophenetic clustering (BIOSYS by Swofford & Selander 1981).

Results and Discussion

Genetic diversity

All of the loci analysed were polymorphic. High levels of polymorphism were observed in the sampled populations (Table 1). A total of 21 alleles were observed of which only one allele was specific to a population. On average, 76% (± 19%) of all loci were polymorphic at the 0.05 level, each locus revealed, on average, 2.84 (± 0.49) alleles and the mean expected heterozygosity, including all loci, was 0.248 (± 0.044). The wide distribution and the high mobility of this butterfly are probably responsible for the important amount of genetic diversity found within each of the sampled populations. These values are characteristic of species having an open population structure (Table 1). In order to maintain this high genetic variability, local effective population sizes must be high to be able to counteract genetic drift and selection. Large effective population sizes probably do not reflect unusually high local population densities, but rather moderate local densities combined with high vagility, as was found in *Pieris napi* (Porter & Geiger 1995).

Table 1 Genetic diversity (GENSERV by Vekemans & Lefèbvre 1997) in *Aglais urticae* populations (in this study) and other species

Populations	*N*	*P*	*A*	H_E
Belgium				
Brabant				
La Hulpe	11	80	2.2	0.206
Hamme-Mille	16	80	2.6	0.273
Genappe	16	60	2.4	0.195
Mean		73.33	2.4	0.224
Standard deviation		11.55	0.2	0.042
Ardenne				
Sart	22	60	2.4	0.294
Bihain	44	80	3.6	0.243
Lienne	20	40	3.0	0.190
Mean		60.00	3.0	0.243
Standard deviation		20.00	0.6	0.052
Holland				
Heteren	32	100	3.4	0.313
Den Burg	37	100	3.2	0.249
Mean		100	3.3	0.248
Standard deviation		0	0.14	0.049
France				
MtVentoux	38	80	2.8	0.265
Mean		76	2.84	0.248
Standard deviation		19	0.49	0.044

N, sample size.
P, percentage of polymorphic loci.
A, mean number of alleles per locus.
H_E, expected panmictic heterozygosity.

Population genetic structure

The average proportion of the genetic variation found between populations was 3.0% (G_{ST} = 0.030; CI = 95% [0.0168, 0.0484]). Again, this value is characteristic of species characterized by an open population structure (Table 2). Gene flow counteracts the effects of genetic drift and selection, resulting in a 'homogenization' of the genetic composition of populations at greater geographical scales. Both genetic drift and local selection increase the genetic diversity found between populations, resulting in G_{ST} values which are higher for species characterized by a closed population structure than for species characterized by an open population structure (where genetic drift and local selection are counterbalanced by gene flow).

Even if most of the total genetic diversity is found within each one of the populations, only those populations sampled within the Brabant region and within The Netherlands were not differentiated (P = 0.0550 and P = 0.3575, respectively).

The Mantel test showed that the matrices representing gene flow and the geographical distances are not significantly correlated (P = 0.8042, r = 0,2437). None of the dendrograms displayed a coherent structure (populations were not grouped in accordance with geographical locations). Lack of correlation between geographical distance and genetic distance is a character of species with high dispersal power (Hastings & Harrison 1994).

As isolation by distance is probably not the cause of the genetic differentiation observed between the populations sampled, an alternative model should be suggested. Extinction and colonization processes may explain the lack of isolation-by-distance patterns observed.

Temporal population genetic structure

Three populations were sampled at different time periods. They are: Bihain (1995–97), Genappe (1996–97) and Hamme-Mille (1996–97). The populations sampled at Bihain are separated by at least four generations, while those sampled at Genappe and Hamme-Mille are separated by at least two generations. None of these populations displayed a significant degree of genetic differentiation from one year to another.

Apart from very small effective population sizes, genetic differentiation through time would occur when: (i) local extinction has taken place (due to significant fluctuations in population size); and (ii) colonization of the empty habitat patch has occurred by individuals from a distant location. It seems that none of the populations we

Table 2 Genetic diversity estimates for different population structures

Species	P	A	H_E	G_{ST}	Source/Reference
Open population structure					
Aglais urticae	76	2.84	0.248	0.030	Present study
Boloria titania	59	2.10	0.157	—	Britten & Brussard (1992)
Pieris napi	37	1.57	0.071	—	Porter & Geiger (1995)
Plutelle xylostelle	—	—	—	0.038 (F_{ST})	Caprio & Tabashnik (1992)
Closed population structure					
Parnassius mnemosyne	38	2.11	0.164	0.075 (F_{ST})	Meglécz *et al*. (1998)
Boloria improba	26	1.30	0.065	—	Britten & Brussard (1991)
Proclossiana eunomia	31	1.38	0.113	0.079 (F_{ST})	G. Nève (unpublished data)

P, percentage of polymorphic loci.
A, mean number of alleles per locus.
H_E, expected panmictic heterozygosity.
G_{ST}, proportion of the total genetic diversity found between populations.

sampled for temporal comparisons fulfilled these criteria. This could be due to the following: (i) the lack of extinction processes during the time period and for those populations that were sampled; (ii) the colonization of empty habitat patches by individuals coming from the same gene pool as those present the year before; or (iii) by selection processes for local conditions which result in the maintenance of certain allele frequencies. It is necessary to allow a test of these hypotheses. Demographic studies are underway to better understand the demographic dynamics responsible for the genetic structure observed.

Conclusions

Populations of *Aglais urticae* showed a high genetic diversity, but only 3.0% of the total genetic diversity is due to a variation between populations. A significant amount of genetic variation was found between the sampled populations but this differentiation was not explained by Wright's model of 'isolation by distance'. We suggest that extinction processes, in which demographic stochasticity plays a major role, create empty habitat patches which are recolonized later by immigrants who could come from as far as a few hundred kilometres away. A combination of high movement rate between neighbouring patches and rare extinction/colonization processes may result in such a genetic population structure not related to the geographical distances between populations. We favour this hypothesis over an island model of population structure because the migration of *A. urticae* individuals is uniform with neither distance nor time. In fact, long-distance migration is somewhat exceptional, is essentially observed in second generation broods and depends entirely on the wind direction and velocity (Roer 1968). This extinction/colonization hypothesis will be verified by in-depth demographic studies and by a more extensive sampling of populations of the whole species distribution.

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The primary aim of this research team is the analysis of the evolutionary and adaptive processes acting on spatially structured populations. Relationships between spatial structure, demography and population genetics are studied at different geographical scales (from isolated populations at the regional scale to populations with a continuous distribution at the regional scale). Relationships between individual behaviour and population structures are also studied. The genetic population structure of *Aglais urticae* will be studied on a Eurasian scale. The evolution of its life-history traits in variable environments will also be analysed.
