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# Population biology of *Proclossiana eunomia* : Preliminary results on morphometric and allozyme variation in Belgian and French populations (Lepidoptera, Nymphalidae)

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**Summary**

Samples of *Proclossiana eunomia* (Esper, 1799) from Belgium and France were analysed by protein electrophoresis and morphometrics. The population from Morvan, central France, where the species was introduced from the Ardennes, is morphologically distinct from its mother population and has lost some alleles, due to a foundation effect. The within-region difference is usually small compared with between-region differences. The validity of the Pyrennean subspecies *P. eunomia ceretanensis* Deslandes, 1930 is confirmed.

**Résumé**

Des échantillons de *Proclossiana eunomia* (Esper, 1799) de France et de Belgique ont été analysés par morphométrie et électrophorèse des protéines. La population du Morvan, où l'espèce a été introduite depuis les Ardennes, est morphologiquement distincte et a perdu des allèles suite à un effet de fondation. Les différences au sein des régions sont en général plus faibles que celles entre les régions. La validité de la sous-espèce pyrénéenne *P. eunomia ceretanensis* Deslandes, 1930 est confirmée.

In Belgium, 81 of the 120 native species of Rhopalocera have shown a significant shrinking of their distribution this century (Baguette *et al.*, 1992). More than half of the Belgian butterfly species are threatened, being in the "endangered", "vulnerable", "rare" or "indetermined" IUCN classes of vulnerability (Baguette & Goffart, 1991). The species more prone to decline are those with strong ecological requirements, and often are linked with specific semi-natural habitats. To address concerns about the future of the declining

species, one needs information, not only of their distribution and habitat requirements, but also of their genetic diversity (Frankel & Soulé, 1980 ; Allendorf, 1983 ; Templeton, 1991).

In order to investigate how this distribution decline may affect the survival of the concerned species, genetic studies have begun independently in France and in Belgium in 1991 on *Proclossiana eunomia* Esper. This species has a very restricted habitat in western Europe : it is found in bogs and unfertilised wet meadows where its only local host plant *Polygonum bistorta* grows (Hackray & Sarlet, 1969 ; Descimon, 1976). The patchiness of this habitat may be seen at different scales, being due both to natural and human factors. Large formerly suitable areas have frequently been fragmented by spruce (*Picea abies*) plantations or by intensively managed and fertilised pasture lands. The local abundance of *P. eunomia* and its strong habitat requirements make this species a good model to investigate how natural and man-made patchiness may influence the genetic structure of natural populations.

How genetically distinct different populations are, and how organised this variation is, are the main themes of our research. The population genetics of *P. eunomia* is currently being studied at different levels : local (within populations, within localities), regional (within regions), and between regions (within the European range of the species). Moreover, as new populations were founded in Morvan in 1970 and 1973, in an area where *P. eunomia* was hitherto absent (Descimon, 1976), the genetics of these populations are investigated and compared with the population of origin of the founder individuals.

## Methods

*P. eunomia* specimens were collected in 1991 in the French Pyrenees, in the two localites where it had been introduced in Morvan (central France), in various localities in Gaume (Southern Belgium) and in the Belgian and French Ardennes, including the locality of origin of the individuals which founded the Morvan populations (Fig. 1). A sample of 206 specimens collected in Morvan in 1977, and in the French Ardennes and the Pyrenees prior to 1991 by H. Descimon was added to the morphometric analysis.

Specimens collected in the field were deep frozen in liquid nitrogen (–196° C) as soon as possible, and kept so until analysis. When thawed in the laboratory, the wings were kept for morphometric analysis, and the body was squashed in a pH 7.1 buffer (15% (w/v) sucrose, 50 mM Tris/HCl pH 7.1, 0.5% (v/v) Triton X-100, drop of Bromophenol Blue as runner marker ; Wynne & Brookes, 1992). Barascud followed the electrophoresis techniques described by Pasteur *et al.* (1987), using horizontal starch gel electrophoresis, and Nève used cellulose acetate electrophoresis methods following Richardson *et al.* (1986) and Wynne *et al.* (1992). Among various allozyme loci studied, the following proved to be polymorphic in the scored populations of *P. eunomia* : Phosphoglucose isomerase (PGI, EC 5.3.1.9), amino aspartate transaminase

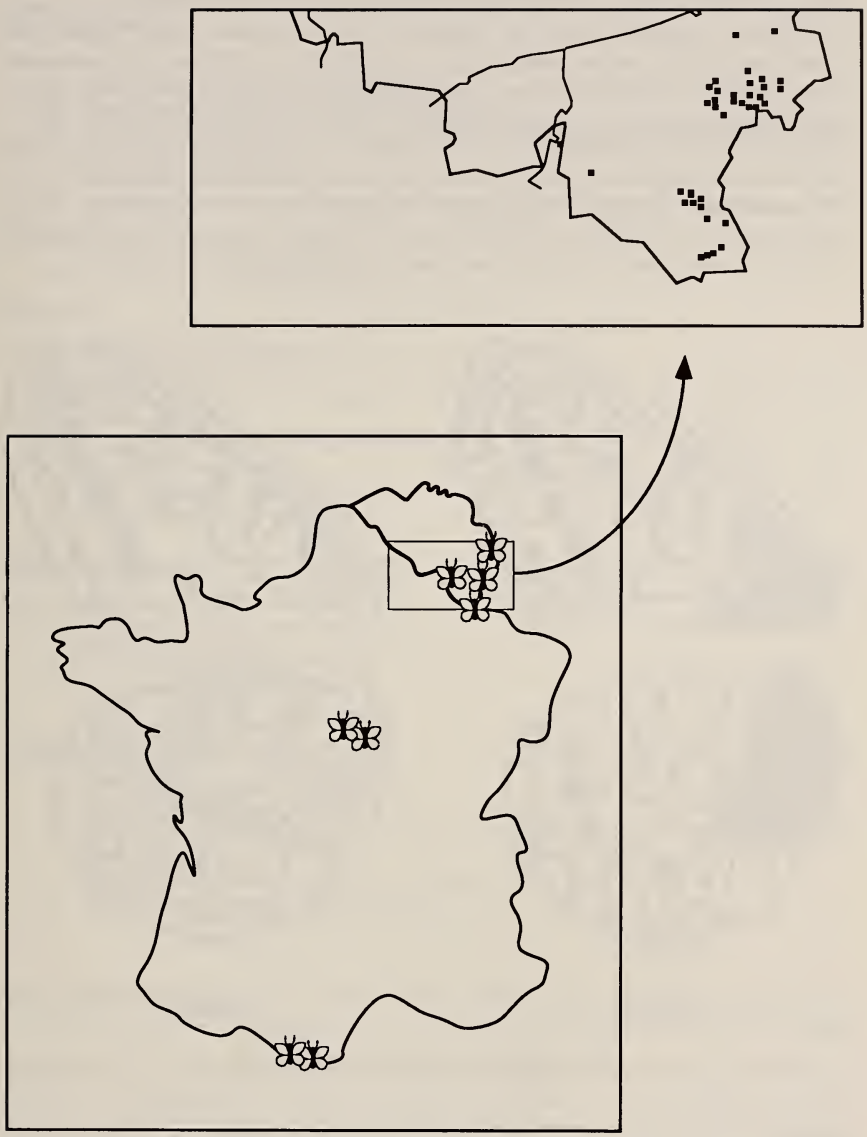

Fig. 1. Distribution of *P. eunomia* samples.

(AAT, EC 2.6.1.1), 6-phosphogluconate deshydrogenase (6PGD, EC 1.1.1.44) and phosphoglucomutase (PGM, EC 2.7.5.1).

Morphometric studies of French specimens were carried out manually, using a binocular microscope and an internal ruler to measure linear dimensions of cells and spots on the wings (Fig. 2, Table 1). For Belgian specimens, an image analyser (description and use described in WINDIG, 1991) was used to take measurements of surface characters (Fig. 2, Table 1). In both cases a principal component analysis was performed on a first data set where 44 and 56 characters respectively were measured on a subsample ; then a set of as few correlated characters as possible was chosen to be measured on all specimens. On the whole, 152 French and 297 Belgian specimens were collected in 1991, of which only a portion has been analysed so far.

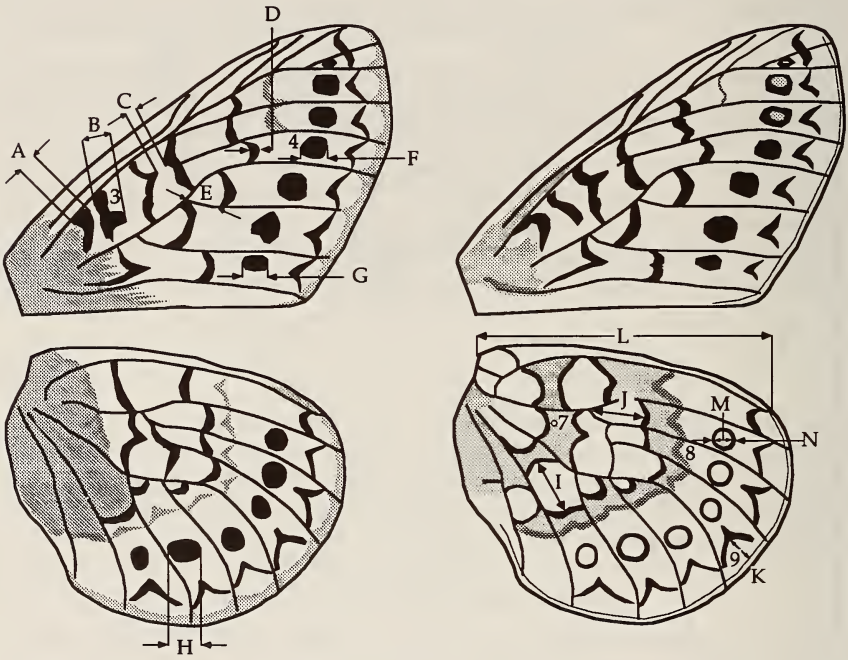


Fig. 2. Wing morphometric characters.

## Results

The morphometric analysis shows that regions within France are well differentiated. The two populations founded in Morvan (central France) with females from the French Ardennes in the early 1970s, already show a significant diffe-

Table 1

List of brief descriptions of morphometric characters measured.
Nomenclature of veins and cells follows HIGGINS & RILEY (1983)

**French specimens**

*Forewing*

A. Maximum width of basal black spot
B. Maximum width of discoidal black spot
C. Distance between the outer edge of the second discoidal spot and the inner edge of the median vein
D. Width of central black spot in s4
E. Distance between the connections of veins 3 and 4 and the basal edge of the spot in s3
F. Diameter of outer spot in s4
G. Diameter of outer spot in s1b

*Hindwing*

H. Diameter of outer spot in s2
I. Length of light "cell" in s1c
J. Distance between the connections of veins 6 and 7 and the inner side of the discal spot in s6
K. Length of submarginal light space in s4
L. "Length" of hind-wing
M. Outer diameter of eyespot in s6
N. Inner diameter of eyespot in s6

**Belgian specimens**

*Upperside of the forewing*

1. Total black surface
2. Total orange surface
3. Area of discoidal black spot
4. Area of outer spot in s4

*Underside of the hindwing*

5. Total black surface
6. Total orange and white surface
7. Contrast index (contrast area x contrast level) of the spot in the orange discal spot of the cell
8. Area of black outer margin of the orange spot of in s5
9. Area of submarginal light space in s4

rence from specimens of their area of origin (MANOVA analysis, $F_{14,44} = 6.36$, $P < 0.001$ for 1977 specimens, $F_{14,157} = 6.60$, $P < 0.001$ for 1991 specimens).

In order to maximise the distance between the regions, a canonical discriminant analysis was performed on the two data sets (Figs 3, 4). In France, on the first two canonical axes, a marked difference was found between the Pyrenean individuals and those from other regions. This result confirms the validity of the Pyrenean subspecies *P. eunomia ceretanensis* Deslandes, 1930. The populations from Morvan were only slightly different from Ardennean populations in 1977, but seem more so in 1991 (Fig. 3). Belgian populations are

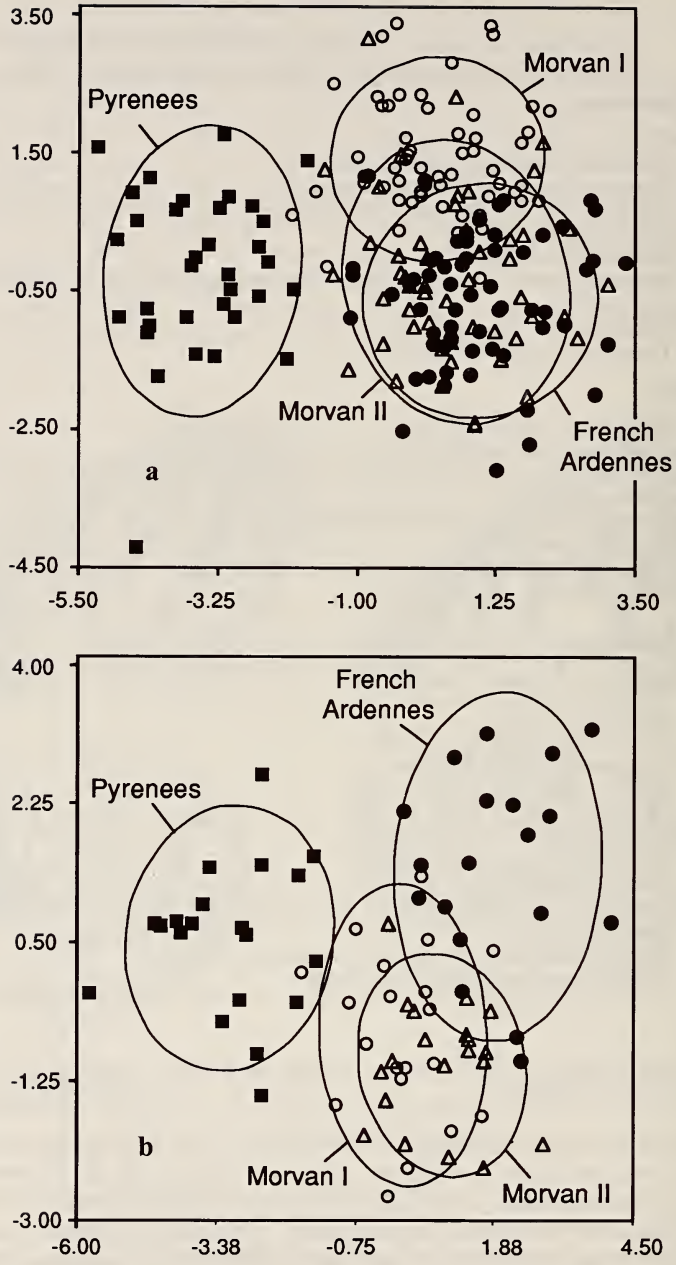


Fig. 3. Canonical discriminant analysis on morphometric characters of French specimens sampled in 1977 (Fig. 3a) and in 1991 (Fig. 3b), projection on the first two axes ; the ellipses show the 80% distribution of the samples of each group. Symbols : Pyrenees = closed squares ; French Ardennes = closed circles ; Saint Brisson (Morvan I) = open circles ; Lavault de Frétoy (Morvan II) = open triangles.

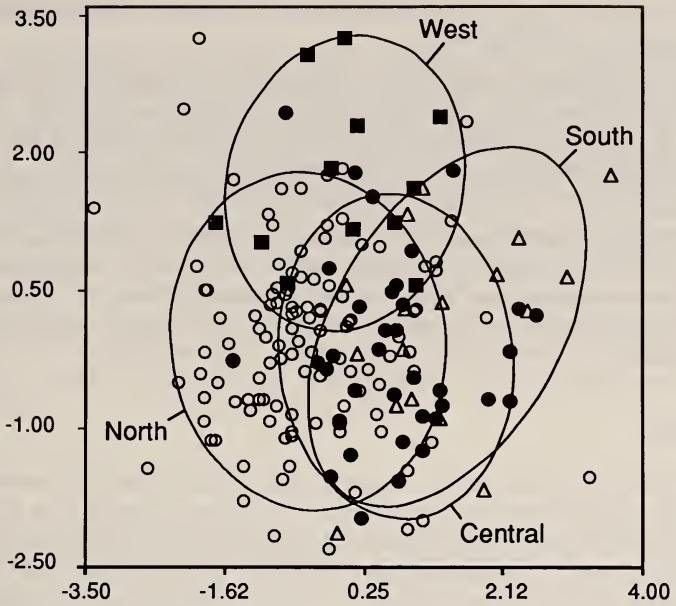


Figure 4. Canonical discriminant analysis on morphometric characters of Belgian specimens sampled in 1991, projection on the first two axes ; the ellipses show the 80% distribution of the samples of each group. Symbols : Graide (West) = closed squares ; Sûre valley (Central) = closed circles ; Plateau des Tailles and Liège Province (North) localities = open circles ; Gaume (South) = open triangles.

Table 2

Frequency of allozyme in French populations, as all studied loci are diallelic, only the frequency of the commonest allele is given.
In each sample 20 individuals were scored

| Region | Locality | Frequency of commonest allele | | |
|---|---|---|---|---|
| | | AAT | PGI | 6PGD |
| Ardennes | Pont Collin | 1.00 | 1.00 | 0.87 |
| Morvan | Saint Brisson | 1.00 | 1.00 | 1.00 |
| | Lavault de Frétoy | 1.00 | 1.00 | 1.00 |
| Pyrenees | Porta | 0.50 | 0.90 | 0.75 |
| | La Tour Cerdane | 0.65 | 0.87 | 0.87 |
| | Porté | 0.85 | 0.97 | 0.85 |

less well differentiated ; they might however display a slight North-South morphological cline (Fig. 4).

Allozyme analysis also confirms the validity of *P. e. ceretanensis*, as populations from the Pyrenees show significant differences with that from the Ardennes (Table 2). In the introduced populations of Morvan, 6PGD has lost the polymorphism present in the mother population at Pont Collin, Ardennes, indicating that the Morvan populations have suffered from a bottleneck effect.

Within Belgian populations, very low genetic differences have been observed so far, as the percentage of the commonest PGM locus varies from 72% to 81% in the 4 regions, and the difference is not significant. Too few data on other loci have been so far collected to allow any further discussion of this genetic data.

## Discussion

The preliminary results of this ongoing study suggest that *P. eunomia* populations show high inter-region variation. This is not surprising, as it has been shown for other species with a disjunct distribution (e.g. *Parnassius mnemosyne* in South France, Napolitano *et al.*, 1988). However, local differentiation and genetic shift of introduced populations *vs* their mother population does not rule out the possibility of selection, which has been proven to occur on the PGM locus in *Maniola jurtina* (Masetti & Scali, 1976), but Carter & Watt (1988) have shown that PGM heterozygosity of *Colias philodice eriphyle* varies with the date of sampling, which suggests a more complicated picture of adaptation of the different PGM alleles to temperature. The morphometric differentiation of the Morvan populations suggests selection pressure, phenotypic plasticity, or both.

Many questions may be raised at this stage in our study. In order to solve at least some of them we plan further work on *P. eunomia*, which will involve (1) pooling both electrophoresis and morphometric data by using the same or compatible methods of investigation in both French and Belgian laboratories ; (2) the collection of more specimens to allow detailed hierarchical analysis of both morphometric and biochemical characters ; (3) the study of further enzymes, in order to validate the estimation of genetic distances between populations and the use of Wright's *F* statistics ; (4) various DNA markers will also be tested, to complement the electrophoresis results ; (5) *P. eunomia* from other regions (e.g. Scandinavia, Bulgaria) will be studied, to investigate its global differentiation and its adaptations to various habitats ; it has been reported to feed on *Polygonum bistorta* in Belgium and France, on *Viola palustris* and possibly *Polygonum viviparum* in Scandinavia (Henriksen & Kreutzer, 1982) and on *Vaccinium uliginosum* and *Andromeda polifolia* in Finland (Marttila *et al.* 1992).

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