Nota lepid. 4 (4) : 125-127 ; 31. XII. 1981 ISSN 0342-7536

Second European Congress of Lepidopterology – Karlsruhe, 1980

Biochemical Taxonomy of the Italian Species of the *Amata phegea* complex (Ctenuchidae, Syntominae)

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The use in taxonomy of multiloci electrophoretic techniques and the evidence they provide for phylogenetic interpretation were pointed out in many recent papers (see for instance Avise, 1974 ; Bullini and Sbordoni, 1980). This approach was applied by us to evaluate the genetic differentiation of the Italian members of the *Amata phegea* complex : *A. phegea* (L.), *A. ragazzii* (Trti), *A. kruegeri* (Ragusa) and *A. marjana* (Stauder).

Electrophoretic analysis was performed on the following enzymes : alcohol dehydrogenase (ADH), octanol dehydrogenase (ODH), sorbitol dehydrogenase (SDH), α-glycerophosphate dehydrogenase (α-GPDH), malate dehydrogenase (MDH), malic enzyme (ME), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6PGDH), aldehyde oxidase (AO), glyceraldehyde-3-phosphate dehydrogenase (G3PDH), xanthine dehydrogenase (XDH), superoxide dismutase (SOD), glutamate-oxaloacetate transaminase (GOT), hexokinase (HK), adenylate kinase (ADK), phosphoglucomutase (PGM), acid phosphatase (ACPH), aldolase (ALD), triose phosphate isomerase (TPI), mannose phosphate isomerase (MPI), esterase (EST), leucine-amide peptidase (LAP) and phosphoglucose isomerase (PGI), plus two non enzymatic proteins (Pt), stained by Coomassie blue. Electrophoretic techniques used were, with minor modifications, those described by Selander *et al.*, 1971, Ayala *et al.*, 1972, Harris and Hopkinson, 1977, Shaw and Prasad, 1970. A total of 34 loci were examined : *Adh-1, Adh-2, Odh, Sdh-1, α-Gpdh, Mdh-1, Mdh-2, Me, Idh-1, Idh-2, 6Pgdh, Ao-1, Ao-2, G3pdh, Xdh, Sod, Got-1, Got-2, Hk-1, Hk-2, Adk, Pgm, Acph-1, Acph-2, Ald-1, Ald-2, Tpi, Mpi, Est-2, Est-5, Lap, Pgi, Pt-1 and Pt-2.*

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Genetic differentiation was calculated from allele frequencies, using Nei's index of standard genetic distance (D), estimating the mean number of allelic substitutions between two taxa (Nei, 1972).

The results obtained can be summarized as follows :

1. the more related species have been found to be *A. phegea* and *A. marjana*, showing a value of average genetic distance ($\bar{D}$) of 0.21, the more differentiated being *A. phegea* and *A. kruegeri* ($\bar{D} = 0.52$) ; these values of $\bar{D}$ well agree with those observed between sibling species belonging to different animal groups (Ayala, 1975 ; Bullini and Sbordoni, 1980) ;
2. *A. kruegeri* and *A. marjana*, considered by Obraztov (1966) and other recent authors as subspecies, have proved to be fully distinct species, the value of D being 0.30 ;
3. a number of diagnostic loci have been found between all the studied species (see Table 1), allowing a sure identification of all specimens, both at the larval and adult stage, including the possible hybrids ;

Table 1
Diagnostic loci between the Italian species of the *Amata phegea* complex (listed, for each possible pair comparison, at the cross of the corresponding row and column)

Species	*A. ragazzii*	*A. kruegeri*	*A. marjana*
A. phegea	*Adh-2, Mdh-1, Mdh-2, Ao-2, Xdh, Hk-2 Pgm, Est-5 Acph-1, Acph-2*	*Adh-1, Mdh-2, Idh-1, 6Pgdh Ao-1, Ao-2 Xdh, Hk-2 Est-5, Acph-1 Ald-2*	*Mdh-2, Pgm Est-5, Ao-2*
A. ragazzii		*Adh-1, Adh-2 Mdh-1, Mdh-2 Idh-1, 6Pgdh Ao-1, Xdh Pgm, Acph-1 Ald-2*	*Adh-2, Mdh-1 Mdh-2, Xdh Pgm, Acph-1 Acph-2*
A. kruegeri			*6Pgdh, Ao-1 Xdh, Acph-1 Acph-2*

4. natural hybrids were found between *A. phegea* and *A. ragazzii*, their frequency ranging from 0 to 0.05 according to the localities (Sbordoni *et al.*, in press) ; no evidence of introgression was detected between the two species ;
5. in spite of the number of *A. phegea* subspecies described in Italy, the populations tested, collected from the Alps to Southern Italy, appear to

be highly homogeneous from the genetic point of view, their values of D ranging from 0.002 to 0.009 ; this suggests that at least a certain amount of gene flow was maintained throughout the Ice Ages, allowed by the considerable eurythermy of this species ;

6. a much higher genetic differentiation was found among *A. ragazzii* geographic populations ; their values of D, ranging from 0.008 to 0.058, are 4-6 times higher than those observed in *A. phegea* ; this is apparently due to interruptions of gene flow occurred during the last Ice Ages, when the surviving populations of *A. ragazzii* were confined in thermophilous refuges ;
7. speciation process in this group appears to be fully allopatric ; according to the Nei's estimation of the time of evolution from values of genetic distance ($t = 10^6 D$, see Nei, 1975), the members of the *A. phegea* complex presumably began to diverge during the Pleistocene, 1 to 2.6 million years ago.

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