REVISION OF THE EUROPEAN SPECIES BELONGING TO THE ECDYONURUS HELVETICUS-GROUP (EPHEMEROPTERA: HEPTAGENIIDAE)

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ABSTRACT

During intensive investigations throughout Europe, specimens of more than eighty populations of the *Ecdyonurus helveticus*-group were sampled. Morphological and biochemical analysis showed the existence of twelve distinct taxa belonging to the *E. helveticus*-complex. The biochemical results, based on the differential mobility of fifteen enzyme-loci, were used to develop a phylogenetic system. This system demonstrated a clear biogeographical cleavage of the *E. helveticus*-complex between an Alpine and an East-European group.

INTRODUCTION

The genus *Ecdyonurus* Eaton, 1868 belongs to the widely distributed family Heptageniidae. The systematic status of the genus has been clearly defined by Zurwerra and Tomka (1985) and is characterized by the existence of lateral pronotum expansions in the larval stage and by distinct lateral and apical sclerites on the penis lobes of the male imago.

The genus *Ecdyonurus* is actually subdivided into two groups : the so-called *E. venosus*- and the *E. helveticus*-groups. Both groups are well differentiated at the imaginal stage by the structure of the apical sclerites of the penis lobes.

In their last revision of the group, Jacob & Braasch (1984) considered the following taxa:

E. carpathicus carpathicus Sowa, 1973
E. carpathicus vitoshensis Jacob and Braasch, 1984
E. epeorides Demoulin, 1955
E. helveticus (Eaton, 1885)
E. krueperi (Stein, 1863)
E. picteti (Meyer-Duer, 1864)
E. siveci Jacob and Braasch, 1984
E. subalpinus (Klápalek, 1907)
E. zelleri (Eaton, 1885)

Since then, two additionnal species from the Alps have been described: *E. alpinus* Hefti, Tomka and Zurwerra, 1987 and *E. parahelveticus* Hefti, Tomka and Zurwerra, 1986. Finally the species *E. austriacus* Kimmins, 1958 which had been incorrectly synonymised with *E. picteti* by Puthz (1975) was elevated as a distinct taxon (Hefti and Tomka, 1986).

During intensive investigations in Austria, France, Germany, Greece, Italy, Romania, Switzerland and Yugoslavia specimens of more than eighty populations of the *E. helveticus*-group were sampled. Due to the wide geographical area over which material was collected, phenotypic variability became readily apparent. This necessitated further characterization by biochemical means in order to separate the various species. For that aim thirty six populations were analysed biochemically using starch gel electrophoresis. All species of the *E. helveticus*-group are included in the following biochemical characterization with the exception of *E. epeorides* for which it was not possible to collect material.

MATERIAL AND METHOD

A detailed list of all the sations prospected in Europe is given in Zurwerra and Tomka (1984), Hefti *et al.* (1986), Hefti and Tomka (1986), Hefti *et al.* (1987) and Hefti and Tomka (1988) for all the taxa of the *E. helveticus*-group. Starch gel electrophoresis was used to investigate the specific electromorph mobilities of fifteen enzyme-loci with the following buffer systems:

Tris-borate-EDTA pH 9 (Ayala *et al.*, 1972) for the enzyme-loci α-glycerophosphate dehydrogenase (α-GPDH), mannose phosphate isomerase (MPI) and retinol dehydrogenase (RDH),

N-(3-amino-propyl)-morpholine citrate pH 7 (Clayton and Tretiak, 1972) for the enzyme-loci aldolase (ALD), glutamate oxaloacetate transaminase (GOT-1 and GOT-2), hexokinase (HK-1 and HK-2), indophenol oxidase (IPO-1 and IPO-2), malate dehydrogenase (MDH-1 and MDH-2) and phosphoglucomutase (PGM). The same buffer system has been used with slight modifications (Zurwerra *et al.*, 1986) at pH 6 for the enzyme-loci adenylate kinase (AK) and arginine phosphokinase (APK).

Details of starch gel electrophoresis method and the evaluation of the data are fully described in Zurwerra *et al.* (1986) and Hefti *et al.* (1988).

RESULTS

The electromorphs distribution of the fifteen enzyme-loci used in the biochemical characterization is presented in Table 1. The α-GPDH, MDH-2, AK, APK, IPO-1 and RDH loci have single electromorph. The α-GPDH and MDH-2 loci are the most conservative enzyme loci with a common value of mobility for all the species investigated. The AK, APK, IPO-1 and RDH loci exhibit in contrast specific patterns of elecromorph mobilities which indicate genetic isolation between species or groups of species. The other enzyme-loci are polymorphic. With one exception, all the taxon present a specific electro-

morphs distribution of the enzyme-loci. This fact demonstrates the distinct systematic status of the taxon investigated electrophoretically. The onliest exception appears between *E. carpathicus carpathicus* and *E. carpathicus vitoshensis*. In this case no genetic isolation has been found on the basis of the enzyme-loci investigated. The two taxon present only intraspecific variations due to the presence of some polymorphic enzyme-loci (ALD, MDH-1). This situation is not in contradiction to their subspecies status.

DISCUSSION

The frequence of the electromorphs (Table 1) has been used for the pairwise correlation of the taxon and the procedure produced an identity matrix defining Nei's genetic distance (Nei, 1972) between two taxa (Fig.1). Then the matrix was clustered according to the unweighted pair-group arithmetic average (UPGMA) method (Ferguson, 1980) to obtain a dendrogram ranging from zero (absolute genetic difference) to one (absolute genetic identity) (Fig. 2). The dendrogram expresses a grouping based on genetic similarities. It reveals a natural biogeographical cleveage inside the *E. helveticus*-group between "Alpine species" (*E. alpinus, E. austriacus, E. helveticus, E. parahelveticus, E. picteti,* and *E. zelleri*) and East-European species (*E. carpathicus carpathicus, E. carpathicus vitoshensis, E. siveci,* and *E. subalpinus*). *E. krueperi* appears in the so-called Alpine group although this does not reflect its phylogenetic affinity.

The elaboration of a phylogenetic system in which *E. krueperi* is correctly assigned can only be achieved when both convergencies and apomorphic characters are clearly recognized. Such a system (Fig. 3) has been constructed after the cladistic definitions of Ax (1984) and was realized as to minimize all the possible convergencies between the species of the *E. helveticus*-group. The biochemical autoapomorphic characters for the ten species investigated electrophoretically are summarized as follows:

(0) Synapomorphy between the Alpine and the East-European species of the *E. helveticus*-group:
- Enzyme IPO-2 (97 or 101) if α-GPDH (100).

The nearest adelphotaxon is the *E. venosus* -group.

(1) Synapomorphy between *E. zelleri* and all the other species of the Alpine group:
- Enzyme IPO-1 (96).

(2) Synapomorphy between the species *E. carpathicus, E. subalpinus* and *E. siveci*:
- Enzyme IPO-2 (101).

(3) Autoapomorphy for the species *E. krueperi*:
- Enzyme ALD (100)
- Enzyme APK (100). Convergence with *E. zelleri*.

(4) Autoapomorphy for the species *E. siveci*:
- Enzyme MPI (102).

- Enzyme GOT-1 (110).

(5) Synapomorphy between E. carpathicus and *E. subalpinus*:
- Enzyme RDH (100).

(6) Autoapomorphy for the species *E. subalpinus*:
- Enzyme GOT-2 (98).

(7) Autoapomorphy for the species *E. carpathicus*:
- Enzyme MPI (101). Convergence with *E. krueperi*.

(8) Autoapomorphy for the species *E. zelleri*:
- Enzyme AK (100).
- Enzyme APK (100). Convergence with *E. krueperi*.

(9) Synapomorphy between the species *E. pictetí* and *E. austriacus*
- Enzyme HK-1 (103/104).
- Enzyme HK-2 (112/113).

(10) Autoapomorphy for the species *E. pictetí*:
- Enzyme PGM (102). Convergence with *E. carpathicus*

(11) Synapomorphy between the species *E. helveticus* and *E. parahelveticus:*
- Enzyme GOT-2 (101).

(12) Autoapomorphy for the species *E. parahelveticus*:
- Enzyme GOT-1 (106). Convergence with *E. pictetí*.

The phylogenetic system obtained in this way clearly separates the Alpine species form the East-European ones. This grouping has also been correlated with some morphological features of the penis lobe of the imagines:

- by all the species of the Alpine group, the outer margin of the apical sclerite is formed by two or more less straight lines and the apical sclerite is divergent and rounded distally forming a club (Fig.4a).
- by all the East-European species, the outer margin of the apical sclerite is regularly curved; the apical sclerite is convergent and more or less pointed distally but it never build a club (Fig. 4b).

These morphological characters have been used to test the phylogenetic incorporation of the species *E. epeorides* on type material (E. Zagora, Mt-Pélion, Greece, Demoulin Collection, Institut Royal des Sciences Naturelles de Belgique). They showed the typical characters related in the East-European group.

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Table 1 : Electromorph frequencies of fifteen enzyme-loci (for abbreviations see text) : enzyme-loci (Enz.), relative mobility index of the electromorphs (Elect.) with their frequencies (%); n = number of specimens analysed.

Taxa		ca.c.	ca.v.	suba.	sive.	krue.	helv.	para.	alpi.	zell.	aust.	pict.
Enz.	Elect.											
	n	6	4	5	32	14	72	28	50	31	15	71
GPDH	100	100	100	100	100	100	100	100	100	100	100	100
	n	6	4	5	36	14	72	28	50	31	15	71
MDH-2	100	100	100	100	100	100	100	100	100	100	100	100
	n	6	4	5	24	14	71	28	50	30	15	71
AK	100	0	0	0	0	0	0	0	0	100	0	0
	106	100	100	100	100	100	100	100	100	0	100	100
	n	6	4	5	24	14	71	28	50	30	15	71
APK	98	100	100	100	100	0	100	100	100	0	100	100
	100	0	0	0	0	100	0	0	0	100	0	0
	n	6	4	5	20	14	71	28	48	29	15	69
IPO-1	96	0	0	0	0	0	100	100	100	100	100	100
	99	100	100	100	100	100	0	0	0	0	0	0
	n	6	4	5	30	14	72	28	50	30	15	71
RDH	100	100	100	100	0	0	0	0	0	0	0	0
	101	0	0	0	100	100	100	100	100	100	100	100
	n	6	4	5	24	14	67	28	47	31	15	71
ALD	95	0	0	100	0	0	100	100	100	100	100	100
	96	33	0	0	100	0	0	0	0	0	0	0
	97	67	100	0	0	0	0	0	0	0	0	0
	100	0	0	0	0	100	0	0	0	0	0	0
	n	6	4	5	22	15	72	28	50	31	16	72
GOT-2	97	0	0	0	0	0	0	0	4	0	0	2
	98	0	0	100	0	0	0	0	0	0	0	0
	99	100	100	0	100	100	0	0	96	100	100	98
	101	0	0	0	0	0	100	100	0	0	0	0
	n	6	4	5	32	14	71	26	38	32	11	77
HK-1	101	0	0	0	0	60	0	0	5	0	0	0
	102	100	100	100	100	40	95	100	95	97	0	0
	103	0	0	0	0	0	5	0	0	3	100	92
	104	0	0	0	0	0	0	0	0	0	0	8
	n	6	4	5	30	14	71	28	51	31	16	71
IPO-2	93	0	0	0	0	0	0	0	4	0	0	0
	97	0	0	0	0	100	96	96	92	100	100	100
	99	0	0	0	0	0	0	4	0	0	0	0
	101	100	100	100	100	0	4	0	4	0	0	0
	n	6	4	5	22	15	62	29	45	31	14	69
MPI	99	0	0	0	0	0	0	0	60	0	0	2
	100	0	0	100	0	0	100	100	36	100	100	98
	101	100	100	0	0	100	0	0	4	0	0	0
	102	0	0	0	100	0	0	0	0	0	0	0
	n	6	4	5	23	14	71	30	48	29	11	69
HK-2	102	0	0	0	0	0	0	7	0	0	0	0
	107	0	0	0	0	57	0	0	0	0	0	0
	108	100	100	100	100	43	100	93	100	100	0	0
	112	0	0	0	0	0	0	0	0	0	33	0
	113	0	0	0	0	0	0	0	0	0	67	100
	n	6	4	5	23	14	76	25	49	29	13	73
GOT-1	100	0	0	0	0	0	5	0	0	0	0	0
	101	0	0	0	0	0	0	0	4	0	0	0
	105	100	100	100	0	100	95	0	96	100	20	0
	106	0	0	0	0	0	0	96	0	0	0	80
	108	0	0	0	0	0	0	4	0	0	80	20
	110	0	0	0	100	0	0	0	0	0	0	0
	n	11	4	10	36	21	105	50	65	37	16	113
MDH-1	96	0	0	0	0	0	0	29	0	0	0	0
	97	0	0	0	0	0	0	4	0	0	0	0
	98	0	0	50	0	50	44	56	25	35	4	46
	100	55	100	50	0	50	45	21	40	40	48	54
	101	0	0	0	0	0	11	0	35	20	48	0
	102	45	0	0	100	0	0	0	0	5	0	0
	n	6	4	5	33	14	74	28	49	43	13	74
PGM	98	0	0	0	0	0	0	0	2	19	0	0
	99	0	0	0	0	0	0	0	0	21	0	0
	100	0	0	0	0	0	0	0	2	0	0	0
	101	0	0	100	0	100	94	100	96	60	100	0
	102	100	100	0	0	0	6	0	0	0	0	96
	103	0	0	0	100	0	0	0	0	0	0	4

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
E. para. (1)	-	.93	.82	.73	.66	.70	.43	.42	.52	.65	.47
E. helv. (2)		-	.90	.75	.67	.77	.51	.51	.59	.72	.48
E. alpi. (3)			-	.79	.70	.81	.59	.59	.67	.69	.56
E. aust. (4)				-	.87	.67	.39	.40	.55	.53	.42
E. pict. (5)					-	.61	.45	.45	.47	.46	.42
E. zell. (6)						-	.45	.44	.64	.56	.42
E. ca.c. (7)							-	.98	.59	.72	.67
E. ca.v. (8)								-	.58	.71	.60
E. krue. (9)									-	.52	.48
E. sub. (10)										-	.54
E. siv. (11)											-

Fig. 1: Identity matrix of relative mobilities of fifteen enzyme-loci between the taxa of the *E. helveticus*-group analysed electrophoretically showing the coefficient of genetic identity (I) between two taxa.

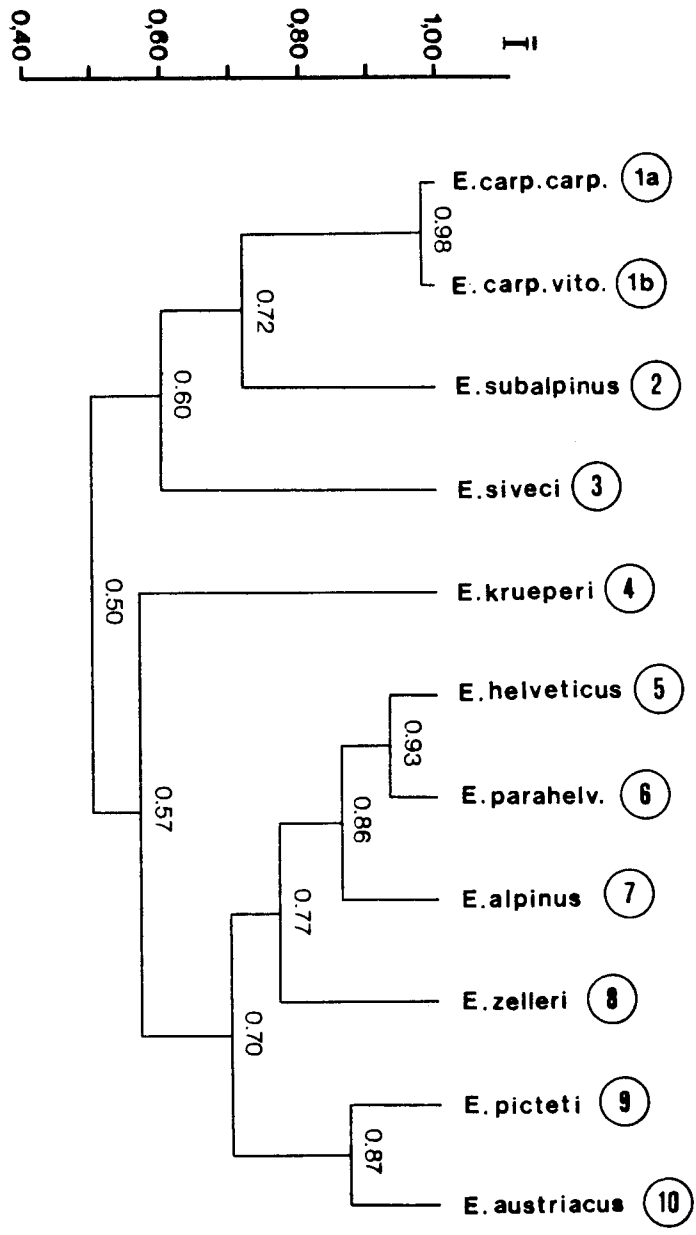

Fig. 2 : Dendrogram of biochemical affinities within the *E. helveticus*-group (except for *E. epeorides*) using the unweighted pair group arithmetic average (UPGMA) clustering method, where I expresses the mean genetic identity.

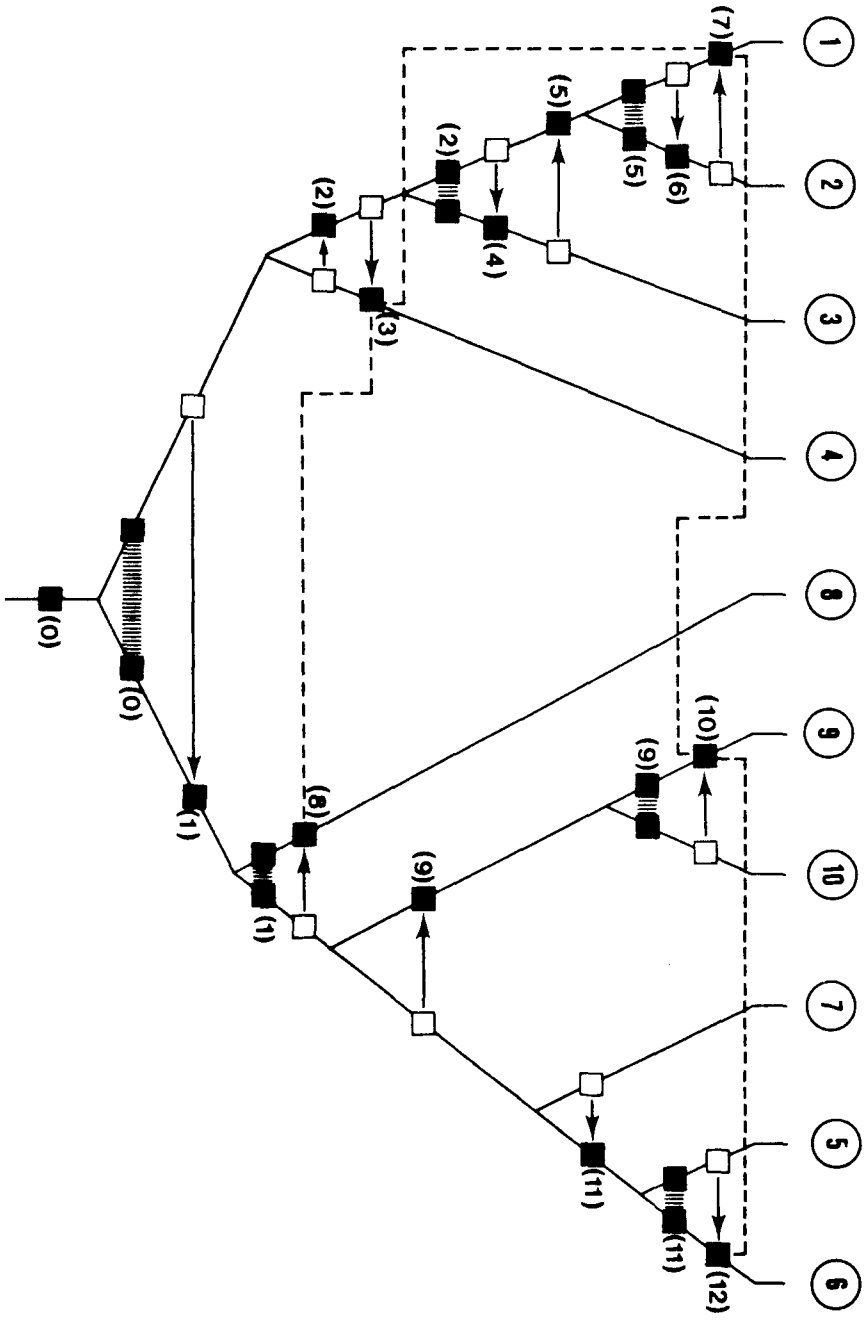

Fig. 3: Cladogram illustrating the phylogeny of the *E. helveticus*-group investigated biochemically.

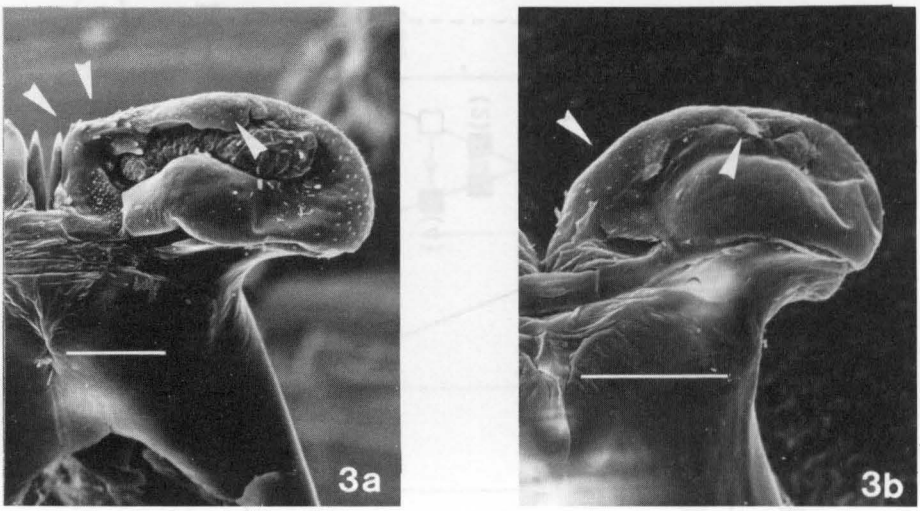

Fig. 4: Penis lobe of a member of the alpine group (3a) and of the east-European group (3b).