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A STUDY ON GENETIC DIFFERENTIATION IN THE MEDITERRANEAN *ISCHNURA* CHARPENTIER (ZYGOPTERA : COENAGRIONIDAE)

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Systematic relationships within the Mediterranean *Inschnura* were investigated through allozyme electrophoretic data. The genetic distances between several populations belonging to *Ischnura elegans, I. genei, I. saharensis, I. graellsii, I. fountainei* and *I. pumilio* were estimated, together with those of two populations of *Erythromma viridulum*, one of *Coenagrion puella* and one of *C. scitulum*. Genetic differentiation levels between populations belonging to *Ischnura* species range widely. It is suggested that *saharensis* and *genei* be regarded as subspecies of *I. elegans*.

INTRODUCTION

In the Mediterranean area, the genus *Ischnura* Charpentier, 1840 is represented by six forms : *I. pumilio* (Charpentier, 1825), *I. fountainei* Morton, 1905 and four basically allopatric forms belonging to the *I. elegans* group (sensy SCHMIDT, 1967) : *I. elegans* (Vander Linden, 1820), that is widely spread in Europe including the Mediterranean area except the Tyrrhenian islands (viz. Sicily, Sardinia, Corsica and their islets) ; *I. genei* (Rambur, 1842), limited to the Tyrrhenian islands ; *I. graellsii* (Rambur, 1842), in the Iberian peninsula and North Africa, and *I. saharensis* Aguesse, 1958, in North Africa south of the Atlas Mountains.

Even though the systematic status of these forms is usually maintained as specific, the systematic relationships within the *elegans* group are still to be cleared. In particular, some questions exist about the specific status of *I. genei*

and *I. saharensis*, that are in turn considered, on a morphological ground, as subspecies of *I. elegans* (AGUESSE, 1968).

The aim of this paper is to contribute to the understanding of the taxonomic status of the Mediterranean *Ischnura*, through the investigation into genetic differentiation patterns in its populations, as evidenced by allozyme polymorphisms. The usefullness of such an approach has been stressed by many authors (e.g., review by THORPE, 1982 ; 1983).

On account of the scanty biochemical information concerning the Odonata, and in particular the Coenagrionidae, besides the six Mediterranean *Ischnura* three other species of the same family have been tested, in order to get a wider view on the genetic differentiation of coenagrionid damselflies.

MATERIAL AND METHODS

Twenty populations of *Ischnura*, of which eighteen of the *elegans* group, two populations of *Erythromma viridulum*, one of *Coenagrion scitulum* and one of *C. puella* were tested for genetic polymorphisms. Species, sample symbols, collecting data and number of assayed individuals are reported in Table I and Figure 1.

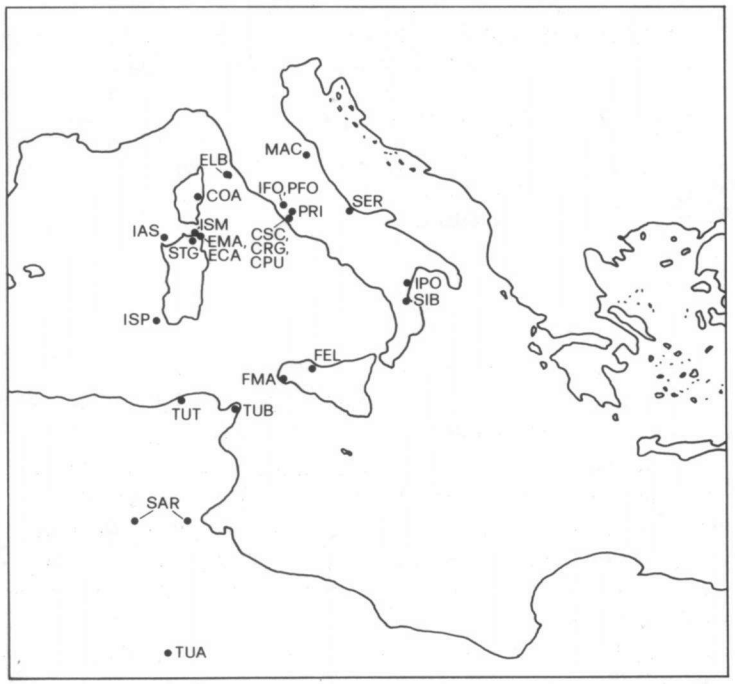

Fig. 1. Map of the central Mediterranean area showing the geographical locations of the sampled populations (black dots). Populations designations refer to Table I.

Table I

Specifications of sampled populations. N refers to sample size.

Species populations	Localities	Collecting dates	N
Ischnura elegans			
MAC	La Pieve, Macerata, Central Italy	Sep. 86	45
IFO	Formello, Rome, Central Italy	Jul. 88	27
CRG	Castelporziano, Rome, Central Italy	Oct. 86	20
PRI	Prima Porta, Rome, Central Italy	Jul. 91	6
SER	Fortore River, Serracapriola, Southern Italy	Sep. 86	30
SIB	Marina di Sibari, Southern Italy	Jul. 86	30
IPO	Policoro, Southern Italy	Jun. 88	33
ELB	Elba Island, Tuscan Archipelago	Jul. 91	6
I. genei			
COA	Aleria and Fiume Oro, Corse	Oct. 86, Jun. 91	35
STG	Porto di Vignola, Sardinia	Oct. 86	14
ISM	S. Maria Island, Sardinia	Aug. 86	31
IAS	Asinara Island, Sardinia	May. 88	50
ISP	S. Pietro Island, Sardinia	Aug. 86, Jun. 87, May. 88	66
FEL	Eleutero river, Palermo, Sicily	Jun. 87	27
FMA	Mazaro river, Mazara del Vallo, Sicily	Oct. 86	18
I. saharensis			
SAR	Nefta and El Hamma, Southern Tunisia	May. 87	21
I. graellsii			
TUT	Tabarka, Northern Tunisia	May.87	48
TUB	Cape Bon, Northern Tunisia	May. 87	22
I. fountainei			
TUA	Bir Amor, Remada, Southern Tunisia	May. 87	35
I. pumilio			
PFO	Formello, Rome, Central Italy	Aug. 88	19
Erythromma viridulum			
EMA	La Maddalena Island, Sardinia	Sep. 87	10
ECA	Caprera Island, Sardinia	Sep. 87	7
Coenagrion scitulum			
CSC	Castelporziano, Rome, Central Italy	Jun. 91	5
C. puella			
CPU	Castelporziano, Rome, Central Italy	Jun. 91	5

Horizontal electrophoresis was carried out on starch gel by using crude homogenates from each whole specimen. All individuals in the study were mature adult males. 16 enzymatic proteins were tested, and 22 zones of activity, encoded by the same number of different loci, were displayed (Table II). Only electrophoretic patterns with a clear resolution were taken into account for the analysis of the genetic structure of the different populations. The procedures used are summarized in Table II.

Estimates of genetic distance (D) were calculated from allozyme data (Nei, 1978, 1987). On the basis of the D values a dendrogram was plotted by using the UPGMA

Table II

A summary of proteins investigated, enzyme codes, procedures used and loci detected in the studied populations of Coenagrionidae. For buffer systems cf. De Matthaeis *et al.*, 1983 and Cobolli Sbordoni *et al.*, 1990.

Codes	Enzymes	E.C. No	Buffers	Staining techniques	Loci
ACO	Aconitase	4.2.1.3.	E	Ward & Beardmore, 1977	Aco-1 ; Aco-2 ; Aco-3
ACPH	Acid phosphatase	3.1.3.2	A	Ayala *et al.*, 1972	Acph
ADA	Adenosine deaminase	3.5.4.4	E	Ward & Beardmore, 1977	Ada
CA	Carbonic anhydrase	4.2.1.1	G	Brewer & Sing, 1970	Ca
CK	Creatine kinase	2.7.3.2	E	Ward & Beardmore, 1977	Ck
EST	Esterase	3.1.1.1	A	Ayala *et al.*, 1972	Est-1 ; Est-2
G6PD	Glucose-6-phosphate dehydrogenase	1.1.1.49	B1	Ayala *et al.*, 1974	G6pd
GOT	Glutamic-oxalacetic transaminase	2.6.1.1	B	Ayala *et al.*, 1975	Got-1 ; Got-2
*a*GPDH	*a*Glyc.-3-phosphate dehydrogenase	1.2.1.12	B	Ayala *et al.*, 1972	*a*Gpdh
HK	Exochinase	2.7.1.1	C	Ayala *et al.*, 1974	Hk-1 ; Hk-2
IDH	Isocitrate dehydrogenase	1.1.1.42	E	Brewer & Sing, 1970	Idh
MPI	Mannose phosphate isomerase	5.3.1.8	F	Harris & Hopkinson, 1978	Mpi
PEP	Peptidase	3.4.11	A	Ward & Beardmore, 1977	Pep-1 ; Pep-2
PGM	Phosphoglucose mutase	2.7.5.1	D	Brewer & Sing, 1970	Pgm
PHI	Phosphoexose isomerase	5.3.1.9	C	Brewer & Sing, 1970	Phi
TO	Tetrazolium oxidase	1.15.1.1	B	Ayala *et al.*, 1972	To

clustering method (SNEATH & SOKAL, 1973). The data analysis was accomplished using the BIOSYS-1 program (SWOFFORD & SELANDER, 1981). The Correspondence Analysis (CA) (BENZECRI, 1973) was used to examine in detail allele frequency relationships among populations within the *elegans* group, using the NTSYS package (ROHLF, 1988).

RESULTS

The genetic distance values and the resulting dendrogram are shown in Table III and Figure 2, respectively. In both these, three different levels of differentiation among populations appear.

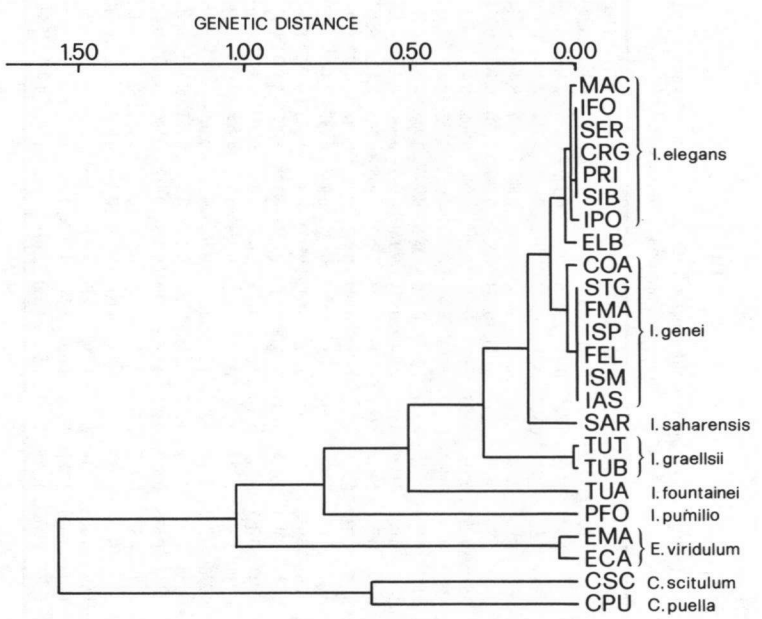

Fig. 2. UPGMA dendrogram based on genetic distances between coenagrionid populations and species.

(1) The first level regards inter-generic distances, that range between D = 1.65 (*Coenagrion* vs *Erythromma*) and D = 1.13 (*Erythromma* vs *Ischnura*).

(2) A lower differentiation level results between congeneric species which are well differentiated for several morphological characters. The respective values range from D = 0.90 (*I. pumilio* vs *I. fountainei*) to D = 0.39 (*I. fountainei* vs *I. saharensis*). Likewise, the genetic distance between *Coenagrion scitulum* and *C. puella* is equal to 0.62.

Table III

Nei's coefficients of genetic identity (upper figure) and genetic distance (lower figure) between the 24 populations sampled. Populations designations as in Tab. I.

	MAC	IFO	CRG	PRI	SER	SIB	IPO	ELB	COA	STG	ISM	IAS	ISP	FEL	FMA	PFO	TUT	TUB	TUA	SAR	EMA	ECA	CSC	CPU
MAC	*****	0.978	0.990	0.987	0.979	0.980	0.973	0.956	0.933	0.910	0.941	0.917	0.920	0.921	0.923	0.451	0.743	0.768	0.612	0.890	0.427	0.349	0.224	0.228
IFO	0.022	*****	0.996	0.994	0.999	0.987	0.987	0.973	0.899	0.885	0.920	0.896	0.893	0.900	0.896	0.467	0.739	0.767	0.587	0.844	0.436	0.359	0.224	0.227
CRG	0.010	0.004	*****	1.000	0.977	0.992	0.986	0.980	0.924	0.904	0.940	0.912	0.911	0.910	0.913	0.464	0.728	0.762	0.586	0.842	0.428	0.348	0.226	0.241
PRI	0.013	0.006	0.000	*****	0.999	0.999	0.980	0.959	0.922	0.877	0.920	0.894	0.891	0.891	0.890	0.433	0.714	0.749	0.572	0.838	0.418	0.342	0.221	0.233
SER	0.022	0.001	0.003	0.001	*****	0.996	0.986	0.965	0.900	0.877	0.913	0.889	0.887	0.889	0.888	0.440	0.722	0.751	0.584	0.837	0.428	0.355	0.222	0.228
SIB	0.020	0.013	0.008	0.001	0.004	*****	0.985	0.953	0.931	0.895	0.929	0.907	0.905	0.906	0.905	0.428	0.714	0.748	0.589	0.867	0.408	0.337	0.210	0.223
IPO	0.027	0.013	0.014	0.020	0.014	0.015	*****	0.983	0.940	0.940	0.963	0.949	0.949	0.953	0.952	0.473	0.751	0.774	0.592	0.866	0.412	0.335	0.202	0.207
ELB	0.045	0.027	0.020	0.042	0.036	0.048	0.017	*****	0.915	0.940	0.967	0.950	0.945	0.947	0.945	0.502	0.766	0.781	0.585	0.801	0.412	0.331	0.217	0.228
COA	0.070	0.106	0.079	0.081	0.105	0.071	0.062	0.088	*****	0.959	0.980	0.974	0.970	0.963	0.968	0.439	0.728	0.756	0.594	0.883	0.361	0.289	0.168	0.189
STG	0.094	0.123	0.101	0.131	0.131	0.111	0.061	0.061	0.042	*****	0.996	0.993	0.997	0.995	1.000	0.478	0.761	0.768	0.612	0.866	0.362	0.292	0.174	0.193
ISM	0.061	0.084	0.062	0.083	0.091	0.074	0.038	0.034	0.021	0.004	*****	0.999	0.998	0.995	0.997	0.487	0.766	0.780	0.609	0.865	0.383	0.306	0.185	0.205
IAS	0.087	0.109	0.093	0.112	0.118	0.098	0.052	0.051	0.026	0.007	0.001	*****	0.998	0.998	0.997	0.485	0.770	0.778	0.601	0.860	0.366	0.293	0.171	0.186
ISP	0.084	0.113	0.093	0.115	0.120	0.100	0.052	0.056	0.031	0.003	0.002	0.002	*****	0.999	1.000	0.485	0.764	0.773	0.601	0.867	0.368	0.293	0.172	0.188
FEL	0.082	0.105	0.094	0.116	0.117	0.099	0.049	0.054	0.037	0.005	0.005	0.002	0.001	*****	1.000	0.490	0.777	0.784	0.618	0.886	0.373	0.296	0.171	0.179
FMA	0.080	0.109	0.091	0.116	0.119	0.100	0.049	0.057	0.033	0.000	0.003	0.003	0.000	0.000	*****	0.487	0.765	0.773	0.603	0.876	0.371	0.297	0.172	0.184
PFO	0.797	0.762	0.769	0.837	0.821	0.849	0.750	0.689	0.824	0.738	0.720	0.723	0.724	0.713	0.720	*****	0.549	0.564	0.404	0.412	0.361	0.293	0.262	0.234
TUT	0.297	0.303	0.317	0.337	0.325	0.338	0.286	0.267	0.317	0.273	0.267	0.261	0.269	0.252	0.268	0.599	*****	0.990	0.658	0.703	0.419	0.351	0.232	0.203
TUB	0.263	0.265	0.272	0.289	0.286	0.290	0.256	0.247	0.280	0.263	0.248	0.251	0.257	0.243	0.257	0.572	0.011	*****	0.660	0.732	0.424	0.345	0.236	0.214
TUA	0.491	0.533	0.535	0.559	0.539	0.529	0.524	0.536	0.522	0.491	0.496	0.509	0.509	0.482	0.506	0.905	0.418	0.415	*****	0.678	0.408	0.344	0.169	0.141
SAR	0.128	0.169	0.172	0.177	0.178	0.143	0.144	0.222	0.125	0.143	0.145	0.151	0.143	0.121	0.132	0.887	0.352	0.312	0.388	*****	0.364	0.296	0.168	0.140
EMA	0.852	0.830	0.848	0.872	0.848	0.897	0.886	0.886	1.018	1.017	0.960	1.006	1.001	0.986	0.993	1.018	0.870	0.859	0.897	1.009	*****	0.941	0.220	0.182
ECA	1.052	1.025	1.055	1.074	1.036	1.086	1.094	1.105	1.241	1.232	1.184	1.226	1.228	1.218	1.214	1.229	1.048	1.063	1.066	1.219	0.060	*****	0.220	0.191
CSC	1.498	1.495	1.485	1.509	1.504	1.559	1.602	1.529	1.781	1.751	1.689	1.765	1.762	1.764	1.761	1.339	1.459	1.443	1.777	1.784	1.513	1.515	*****	0.536
CPU	1.479	1.484	1.422	1.459	1.478	1.501	1.574	1.477	1.668	1.645	1.585	1.680	1.671	1.722	1.692	1.452	1.597	1.542	1.959	1.966	1.652	1.654	0.624	*****

(3) The differentiation levels between the populations belonging to the forms in the *elegans* group are much lower. Only the *graellsii* populations (TUT, TUB) appear to be genetically well differentiated, also on account of the presence of alternative alleles in three loci, compared to the other forms. The genetic distances between the *graellsii* populations and all other populations of the *elegans* group range from D = 0.35 (TUT vs SAR) to D = 0.24 (TUT vs FEL). The values exhibited by the remaining populations are much lower. In detail, average D is 0.15 (R = 0.22-0.12) between *saharensis* and *genei*, 0.16 (R = 0.18-0.13) between *saharensis* and *elegans* and 0.08 (R = 0.13-0.03) between *elegans* and *genei*.

The D values between different populations of the same forms are even lower, ranging from 0.04 to 0.00 in *genei* and from 0.05 to 0.00 in *elegans* (Tab. III). It is to be outlined that the range of the genetic distance values between *elegans* and *genei* partially overlaps those between populations within the single forms.

The CA plot (Fig. 3) offers a deeper insight into the genetic relationships among the populations of *saharensis, genei* and *elegans*. The higher similarity of the latter two is represented here by the thick grouping of all their populations around the axis origin. However, some separation of two contiguous groups, each consisting of conspecific populations, is evident. This graph also shows both the relative isolation of *saharensis* from the other two forms and, considering the order of the populations along the first axis, its relatively closer similarity to *genei* than to *elegans*.

DISCUSSION

In our data, the genetic differentiation levels between the genera *Ischnura, Coenagrion* and *Erythromma* match those reported in the literature for other animal groups (THORPE, 1983). This is also valid for congeneric species within the genus *Coenagrion* and, as far as *Ischnura* is concerned, between *pumilio, fountainei* and the *elegans* groups as a whole. In particular, our values are similar to those obtained by MAIBACH (1985) for the European *Calopteryx*. In contrast, the genetic distance values within the *elegans* group raise some questions. The form *graellsii* shows values which match those relative to semispecies or incipient species in other insects (AYALA, 1983). This form also shows male terminalia markedly different from those of the other forms in the *elegans* group (cf. DUMONT, 1977). However, as mentioned above, its (moderate) genetic differentiation also results from three alternative alleles. Since, according with COYNE (1992) "... changes in at least two genes are usually required for speciation ...", we confirm the taxonomic status of *I. graellsii* as specific. In fact, even though *I. graellsii* and *I. elegans* are sympatric in some areas in the Iberian Peninsula (CORDERO, 1989, and pers. comm.)

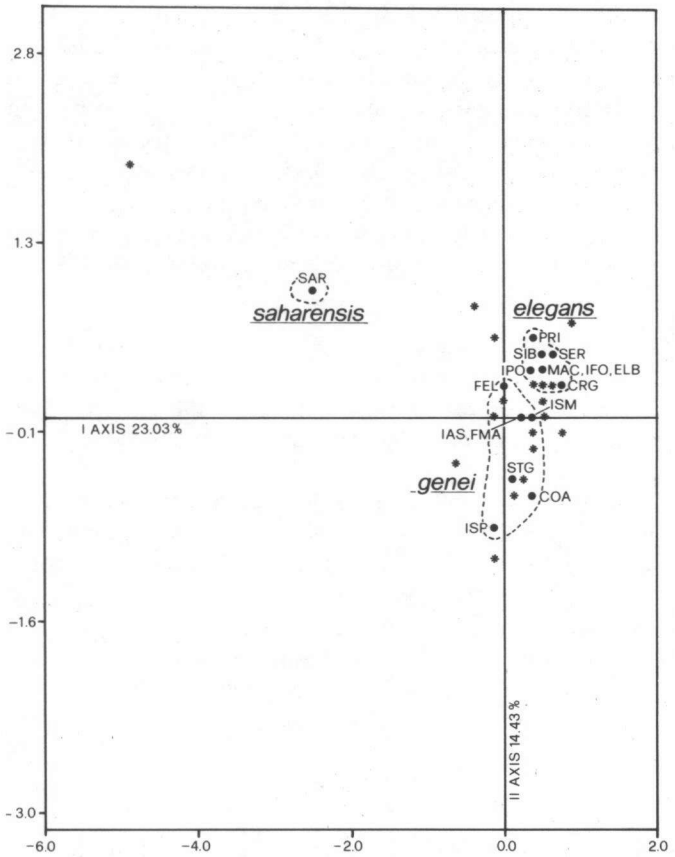

Fig. 3. A two dimensional plot of populations of *Ischnura elegans, I. genei* and *I. saharensis* resulting from correspondence analysis of allele frequencies. Black dots denote populations and asterisks alleles at enzyme loci.

and interspecific pairing may occurr in the laboratory, their crossings are sterile (CORDERO, 1989).

On the other hand, the very low genetic distances between the populations of the other three forms in the *elegans* group do not support their inclusion into discrete specific taxa. Indeed AGUESSE (1968), on the basis of the penis morphology, regards both *genei* and *saharensis* as subspecies of *I. elegans*.

Anyway, the genetic distances between the forms *genei, saharensis* and *elegans* do not parallel the morphological differences in the male abdominal appendages. Basing on the latter, *genei* looks much closer to *saharensis* than to *elegans* (cf. also DUMONT, 1977 ; 1991), while the reverse appears in our dendrogram (Fig. 2).

In conclusion, on the basis of our biochemical data, we must regard *I. pumilio, I. fountainei* and *I. elegans* as good species and agree with SCHMIDT's (1967) opinion that the genus *Ischnura* may be divided into species groups of which the former represent the typical species (the fourth group, *I. senegalensis*, is not present in the Mediterranean area). Within the *elegans* group, *I. graellsii* can be considered a good species, while *genei* and *saharensis* probably represent geographical races of *I. elegans*. The closer genetic similarity between the forms *elegans* and *genei* might be due to some gene flow in spite of their allopatric distribution. As a matter of fact, specimens of both *I. genei* and *I. elegans* have been occasionally collected in the islands of Giglio and Elba (Tuscan Archipelago) (CAPRA, 1976 ; TERZANI, 1983) and recently in Sardinia (BURMEISTER, 1989). The finding of a tandem pair of *I. genei* in flight well offshore (CAPRA, 1976) is a good hint of the potential of this damselfly for dispersal between the large Tyrrenian island and the mainland of Italy, eventually enhanced by the "bridge" represented by numerous liner ships connecting these islands to the continent. Nevertheless, since a low genetic distance may sometimes exist even between good species (AYALA 1983), investigations on the degree of interfecundity (if any) between *elegans* and *genei* in the field and experimental crossing in the laboratory would be needed for the best resolution of this critical point.

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