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Genetic Variability and Taxonomical Considerations about Six Species of European Cardueline Finches (Aves, Passeriformes)

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ABSTRACT. Electrophoretic patterns of seven enzyme systems and two nonenzymatic proteins, albumin and haemoglobin, representing 13 loci, have been investigated in six species of cardueline finches with Palearctic distribution. A new locus, SOD-3, has been characterized in this subfamily. The two nonenzymatic proteins turned out to be the best genetic markers. From the analysis of the genetic distances it emerges that the mean values between species are higher than those reported for other groups of birds. However, the same results were obtained for other species of the same subfamily native to North America. In both cases this result is probably due to a classification that disagrees with the real cardueline phylogeny. In the present study, the attribution of the Greenfinch to the genus *Carduelis* raises the mean value of distance between species. COMP BIOCHEM PHYSIOL 118B;4:771–775, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Allozymes, cardueline finches, electrophoresis, genetic distance, *Carduelis*, *Coccothraustes*, *Serinus*

INTRODUCTION

Systematic and phylogenetic relationships within the subfamily of cardueline finches (Aves, Passeriformes) is one of the most controversial topics regarding birds. Limits of species and genera are not yet clear (21), particularly among Old World forms (18) and are based chiefly on morphological and anatomical similarities, common behavioral traits, and immunological affinities (6,7,12,16,17,22,27,29,30). The most recent taxonomic lists for this taxon are those of Sibley and Monroe (26) and Sibley and Ahlquist (25), who regard the Carduelinae as a tribe containing 135 species in 20 genera, while Clement *et al.* (8) refer to 125 species in 20 genera, one of which is, however, different from those of the previous authors. A contribution to solve this taxonomic question may come from electrophoretic analysis of enzyme systems (isozymes and allozymes; 10), a method whose utility in avian studies is described in (19). Marten and Johnson (18) have already carried out an electrophoretic survey of genetic variation in 14 species of North American cardueline finches. To extend the knowledge about the cardueline group and to contribute to the awareness of the genetic variability and the phylogenetic relationships within this subfamily, as suggested by Marten and Johnson (18), six species of European Carduelinae (belonging to the genera: *Carduelis*, *Coccothraustes*, and *Serinus*), never tested before, were examined.

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MATERIALS AND METHODS

Animals

Adult individuals of Goldfinch (*Carduelis carduelis*; $N = 43$), Linnet (*C. cannabina*; $N = 31$), Greenfinch (*C. chloris*; $N = 25$), Serin (*Serinus serinus*; $N = 15$), and Hawfinch (*Coccothraustes coccothraustes*; $N = 8$), captured in Sicily (Italy) in winter 1992/93, and individuals of the domestic form of Canary (*S. canario*; $N = 25$) were examined.

Sample Preparation

Blood samples were taken from the brachial vein of captured birds with heparinized tubes. After the sample collection the tubes were stored at 4°C and the birds, after the necessary treatment, were immediately ringed and released.

Within 8 hr from collection, the blood samples were taken into the laboratory and then centrifuged at 15,000 rpm for 10 min to separate the red-cells fraction from the plasma. Afterwards the red cells were washed twice in a 0.85% saline solution, lysed with distilled water and finally centrifuged at 10,000 rpm for 15 min [cf. (13)]. Both the plasma and the red cells fraction were diluted 1:1.5 and 1:9, respectively, with distilled water before electrophoresis. On the contrary, to detect albumin, which is found in characteristically high concentrations (23), the plasma was di-

TABLE 1. Distribution of the electromorphs and, at the polymorphic loci, allelic frequencies in parentheses in six species of Cardueline finches

	Monomorphic in all the species				Monomorphic but characteristic of one species or a group of species							Monomorphic in some species and polymorphic in the others	
	MDH	E	LDH-1	Hb-1	SOD-1	SOD-2	SOD-3	PGM-1	PGM-2	SDH	Hb-2	Alb	GPI
Carduelis carduelis (N = 43)	a	a	a	a	b	b	b	a	a	b	c	b	a
Carduelis cannabina (N = 31)	a	a	a	a	b	b	b	a	a	b	a	e	c (.95) d (.05)
Carduelis chloris (N = 25)	a	a	a	a	a	a	a	a	a	b	d	a (.67) f (.33)	a
Coccothraustes coccothraustes (N = 8)	a	a	a	a	a	a	a	b	b	a	e	a (.75) f (.25)	a
Serinus serinus (N = 15)	a	a	a	a	b	b	b	a	a	b	b	c	a (.71) b (.29)
Serinus canaria (N = 25)	a	a	a	a	b	b	b	a	a	b	b	d	a

luted 1:15 with distilled water. We also used blood samples from hybrid individuals, born in captivity from females of *S. canaria* interbred with males of *C. carduelis* (5 hybrids), *C. chloris* (3 hybrids) and *S. serinus* (4 hybrids), respectively.

Staining

By using electrophoresis on polyacrylamide gel slab (PAGE) following Davis (1), we analyzed the genetic variability of seven enzyme systems: malate dehydrogenase (MDH; E.C. 1.1.1.37), cholinesterase (ChE; E.C. 3.1.1.8), lactate dehydrogenase (LDH; E.C. 1.1.1.27), phosphoglucomutase (PGM; E.C. 5.4.2.2), sorbitol dehydrogenase (SDH; E.C. 1.1.1.14), superoxide dismutase (SOD; E.C.1.15.1.1); and glucose-6-phosphate isomerase (GPI; E.C. 5.3.1.9). Two nonenzymatic proteins, albumin (Alb) and haemoglobin (Hb), were also analysed. The staining procedures described by Harris and Hopkinson (15), Shaw and Prasad (24) and Richardson *et al.* (23), were adapted and used here.

Statistics

Different electrophoretic mobility was considered as being a product of different alleles [cf. (2,3)]. On the basis of their electrophoretic mobility, for each locus the alleles received an alphabetic designation, "a" denoting the most cathodal form. The computer programme BIOSYS-1 (28) was used to compute allelic frequencies and to convert them to genetic distance (20).

RESULTS AND DISCUSSION

Genetic Variability

The seven enzyme systems and the two nonenzymatic proteins examined in the six species showed 13 presumptive loci. Four of these were monomorphic and presented the same electrophoretic mobility in all the species (MDH, ChE, LDH-1, Hb1), seven were monomorphic, but characteristic of one species or a group of species (SOD-1, SOD-2, SOD-3, PGM-1, PGM-2, SDH, Hb2), and only two showed a monomorphic pattern in some species and polymorphism in the others (Alb, GPI) (Table 1). The use of hybrid individuals helped to clarify complex polymorphic patterns. We did not analyze two additional loci (EST, E.C. 3.1.1.1.; LDH-2), because their patterns were uninterpretable, the former expressing six loci and being very polymorphic, and the latter being not very clear in blood samples.

Marten and Johnson (18) were the first to study enzymatic variants among 15 species of Cardueline, all but one (*C. carduelis*) living in North America. The comparison of our results with theirs showed that seven loci were in common. Allelic variants of these loci seemed to be the same as regards LDH-1 and MDH-1 (which appeared monomorphic for all the species), SOD-1 and SDH (which appeared monomorphic but fixed for alternative alleles), and GPI

TABLE 2. Matrix of Nei's genetic distances between six species of Carduelinae finches

Species	*C. carduelis*	*C. cannabina*	*C. chloris*	*C. coccothraustes*	*S. serinus*
Carduelis cannabina	0.018	—			
Carduelis chloris	0.468	0.599	—		
Coccothraustes coccothraustes	0.940	1.161	0.382	—	
Serinus serinus	0.178	0.243	0.489	0.985	—
Serinus canaria	0.167	0.260	0.468	0.940	0.088

(which appeared polymorphic in many species). On the contrary, differences appeared in SOD-2, PGM-2 and PGM-1 patterns. The former two loci seemed to Marten and Johnson (18) to be monomorphic and equal for all the American species, while the latter showed many polymorphisms within the species. Conversely, our results showed that all the three loci were monomorphic but fixed at alternative alleles. Moreover in C. *carduelis*, the only species common to both studies, we did not find PGM-1 to be polymorphic. This difference could be due to the use of different tissues or different populations. Although SOD-3 has been described in *Fringilla coelebs*, a confamiliar species (4) for the first time we characterized this enzyme in the cardueline subfamily. Its electrophoretic pattern appeared similar to those of SOD-1 and SOD-2. High similarity among the species was indicated by the common loci, while differences seemed to be bound mainly to allelic assortments. Hemoglobin and Alb proved to be good genetic markers (Table 1). The electrophoretic patterns showed that the distance between the two characteristic bands of Hb is species-specific. However, this close connection is not due to the Hb1, which appeared identical among the species examined, but to the different electrophoretic mobility of Hb2. Only in the two species of the genus *Serinus* does the mobility of Hb2 seem to be identical. Conterio and Mainardi (9) have already shown by means of paper electrophoresis of eight species of Carduelinae (including those examined in the present study) that the percent ratio of Hb1 and Hb2 was characteristic for every species. Also, Alb showed typical bands for the species examined except for C. *coccothraustes* and C. *chloris*, which have two identical alleles with different frequencies.

Taxonomical Relationships

To examine the genetic distances (D) the method of Nei (20) using allelic frequencies (Table 2) was adopted. The D values between those species belonging to the genus *Serinus* and those belonging to the genus *Carduelis* are just as great as the distance values between species that are held to belong to this second genus. This result is concordant with data obtained by Groth (14) on the same two genera using mitochondrial DNA analysis.

The calculated values of D ranged from 0.088 to 1.161 and were not very different from those reported by Marten and Johnson (18) for other species of the same subfamily. However, their mean values remained higher than those reported on average by Barrowclough (5) for other groups of birds (Table 3).

In our study, this is due to the Greenfinch (*Carduelis chloris*): the values of genetic distance of this species from the other congeneric species examined were high and similar to those usually calculated among different genera. It is probable also that Marten and Johnson (18) reached the same result in consequence of the attribution of some species into the wrong genus.

From the analysis of genetic distances (Table 2), it emerges that every single D value is higher than the maximum value of genetic distance between species reported by Barrowclough (5), except for *Serinus serinus* and *Serinus canaria*.

In spite of high taxonomic confusion that reigns in the subfamily of Carduelinae, our preliminary results suggest, as Arrigoni degli Oddi (1) already suspected, that every species of European Carduelinae should stay in a different genus from the others.

So it would be necessary, as Groth (14) has also suggested, to carry out a deep revision of this taxon with molecular methods, seeing as the results thus obtained seem to converge.

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TABLE 3. Comparison between the mean genetic distances among sample from species differentiated at several taxonomic levels

	Barrowclough, 1980				Marten & Johnson, 1986				This study	
	Sub-species	Species	Genera	Families	Sub-species	Species	Genera	Families	Species	Genera
Mean genetic distance	0.0048	0.044	0.2136	0.6829	0.0048	0.1739	0.5209	0.9239	0.3538	0.5648
Standard error	0.0005	0.0026	0.0141	0.0304	0.0021	0.0206	0.0143	0.0303	0.1128	0.11147
Standard deviation	0.0049	0.0221	0.1659	0.197	0.0042	0.1183	0.1655	0.1321	0.2255	0.3697
Range	−0.0014–0.0214	0.0078–0.1267	0.0126–1.2140	0.3365–1.2140	0.000–0.010	0.028–0.527	0.193–0.883	0.622–1.144	0.088–0.599	0.167–1.161
Sample size	86	71	139	42	4	33	134	19	4	11

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