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INTERSPECIFIC RELATIONSHIPS IN THE *Cardini* GROUP OF *Drosophila* STUDIED BY ELECTROPHORESIS

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ABSTRACT

The electrophoretic patterns of six adult enzyme systems and of total pupal proteins were examined in a comparative study of 13 *Drosophila* species of the *cardini* group. Pair comparison among the species showed a level of genetic similarity of approximately 0.50 among the species of the *arawakana-caribiana-nigrodunni* complex. *D. antillea*, which belongs to this group, was analyzed only for esterases. A pattern extremely similar to that of the others was obtained. Similarly, the triad formed by *D. cardinoides*, *D. procardinoides* and *D. parthenogenetica* showed a mean similarity index of 0.43. These results confirm conclusions reached by other investigators based on reproductive affinity and cytology. However, *dunni* and *belladunni*, which belong to a complex of three insular species with close cytological similarity, only showed 0.15 similarity by the electrophoretic criterion.

INTRODUCTION

The *cardini* group comprises 16 species: 8 insular species found in the Greater and Lesser Antilles, and 8 species distributed in populations of varying size throughout tropical continental America (Heed and Russel, 1971). The phylogenetic relationships among the species in this group were established

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by hybridization tests (Stalker, 1953; Heed and Krishnamurthy, 1959; Futch, 1962; Heed, 1962) and by cytological studies (Heed and Russel, 1971).

Several investigators have used electrophoretic isoenzyme analysis to evaluate the degree of genetic similarity among species (Hubby and Trockmorton, 1968; Nair *et al.*, 1971; Yang *et al.*, 1972; Ayala *et al.*, 1975; Johnson *et al.*, 1975; Richardson *et al.*, 1975; Sene and Carson, 1977; Napp and Brncic, 1978). The objective of the present study was to attempt to establish phylogenetic relationships among 13 *Drosophila* species of the *cardini* group, as well as evaluate the degree of genetic modification due to mutation in the structural genes involved in the speciation process in this group.

MATERIAL AND METHODS

The electrophoretic patterns of six adult enzyme systems and of total pupal protein were analyzed in 13 species of the *cardini* group. A minimum of one and a maximum of nine stocks were examined for each species (Table I). Further information on the origin and maintenance of a large part of the stocks is found in Heed and Russel (1971).

The samples were prepared for electrophoresis by individually homogenizing 8 to 25 adult flies aged 8 days. Brown pupae were utilized for total protein. Starch gel electrophoresis in a discontinuous system of buffers was used (Poulik, 1957). The field intensity was approximately 10 V/cm for 3 to 4 hours until the migrating front reached 10 cm from the point of application. The staining methods used were similar to those described by Ayala *et al.* (1972), with small modifications.

Table I - Number and origin of laboratory stocks analyzed in each species.

Species	Collection Number	Locality
D. polymorpha (PO)	H 330.12	Trinidad
	H 333.32	Trinidad
	W 19	Venezuela
	H 186.49	El Recuerdo, Colombia
	134	Restinga, RS
D. neomorpha (NO)	H 51.10	Honduras
D. neocardini (NE)	H 339.1	Angra dos Reis, RJ
	H 340.7	Mogi das Cruzes
	77	Ponta do Caximbo, RS

continued

Table I - continued

Species	Collection Number	Locality
D. cardinoides (CA)	H 442.7	Colombia
	H 234.1	Trinidad
	2253.2	Mexico
	H 188.5	Colombia
	H 27.9	El Salvador
	H 191.21	Colombia
	505.14	Venezuela
	H 336.10	Belém
	51	Ponta do Caximbo, RS
D. procardinoides (PR)	H 346.8	Bolivia
D. parthenogenetica (PA)	H 50.25	Honduras
	A 33.7	Mexico
D. bedicheki (BD)	I TR.5	Trinidad
D. cardini (CR)	WM 114.9	Mexico
	A 52.7	Mexico
	H 332.18	Trinidad
	H 260.26	Puerto Rico
	H 355.8	Jamaica
	2395.6	Pisco, Peru
	2548.1	Chile
	H 336.31	Brazil
D. acutilabella (AC)	2380.2	Cuba
	2350.2	Cuba
	A 169.1	Florida
	H 355.3	Jamaica
	H 136.8	Jamaica
	H 413.7	Haiti
D. dunni (DU)	H 253.5	St. Thomas
	H 129.4	Puerto Rico
D. belladunni (BE)	H 356.3	Jamaica
D. similis (SI)	H 240.1	St. Vincent
D. arawakana (AR)	H 126.1	St. Kitts
	A 112	Mont Serrat
	H 252.7	Guadaloupe
D. caribiana (CB)	H 248.1	Martinique
D. antillea (AN)	H 122.1	St. Lucia
D. nigrodunni (NI)	H 116.2	Barbados
	H 249.1	Barbados

RESULTS

The electrophoretic patterns of six adult enzyme systems and of total pupal proteins for the different species studied are shown in the diagrams in Figure 1. The diagrams show only the bands utilized for the study of the taxonomic relationships among the species. The bands are numbered starting from the anodic band of highest mobility. The first two colums in each diagram show the total number of variants in one system found for all species studied.

The coefficients of genetic similarity were calculated by comparing the species pair by pair on the basis of identical bands found in relation to the number of bands found for the two species. As reported by Nair *et al.*, (1971), this method gives results comparable to those obtained by the method of Rogers (1972).

Table II gives the coefficients of similarity calculated for the 13 species in the group, with a total of 78 pair-by-pair comparisons. *D. antillea, D. similis* and *D. bedichecki* were not included since each was studied only in terms of one or two enzymatic systems. For comparison purposes, the species were grouped on the basis of the cytological analysis done by Heed and Russel (1971). According to these investigators, the 16 species in the *cardini* group are divided into two subgroups: *cardini,* consisting of 8 species distributed throughout continental South and Central America, plus *D. acutilabella,* although this is an insular species. The second subgroup, *dunni,* consists of seven insular species living in Porto Rico, Jamaica and the Lesser Antilles: *D. belladunni, D. similis, D. dunni, D. arawakana, D. antillea, D. caribiana,* and *D. nigrodunni.* On the basis of isozymic data obtained within the two subgroups, the estimates of genetic similarity are comparable. The coefficients calculated for the species within the *cardini* subgroup had a mean value of 21.6%, and those for the species in the *dunni* subgroup had a mean value of 23.3%. When species belonging to one or the other subgroup were compared, the mean coefficient of similarity was 18.2%. The highest coefficient of similarity was that between *D. procardinoides* and *D. parthenogenetica* (61.5%), and the lowest was that between *D. belladunni* and *D. cardini* (3.3%). In turn, *D. cardini* was more related to *D. parthenogenetica* and *D. cardinoides,* showing an isozymic similarity of 36.7% and 41.2% with these species, respectively.

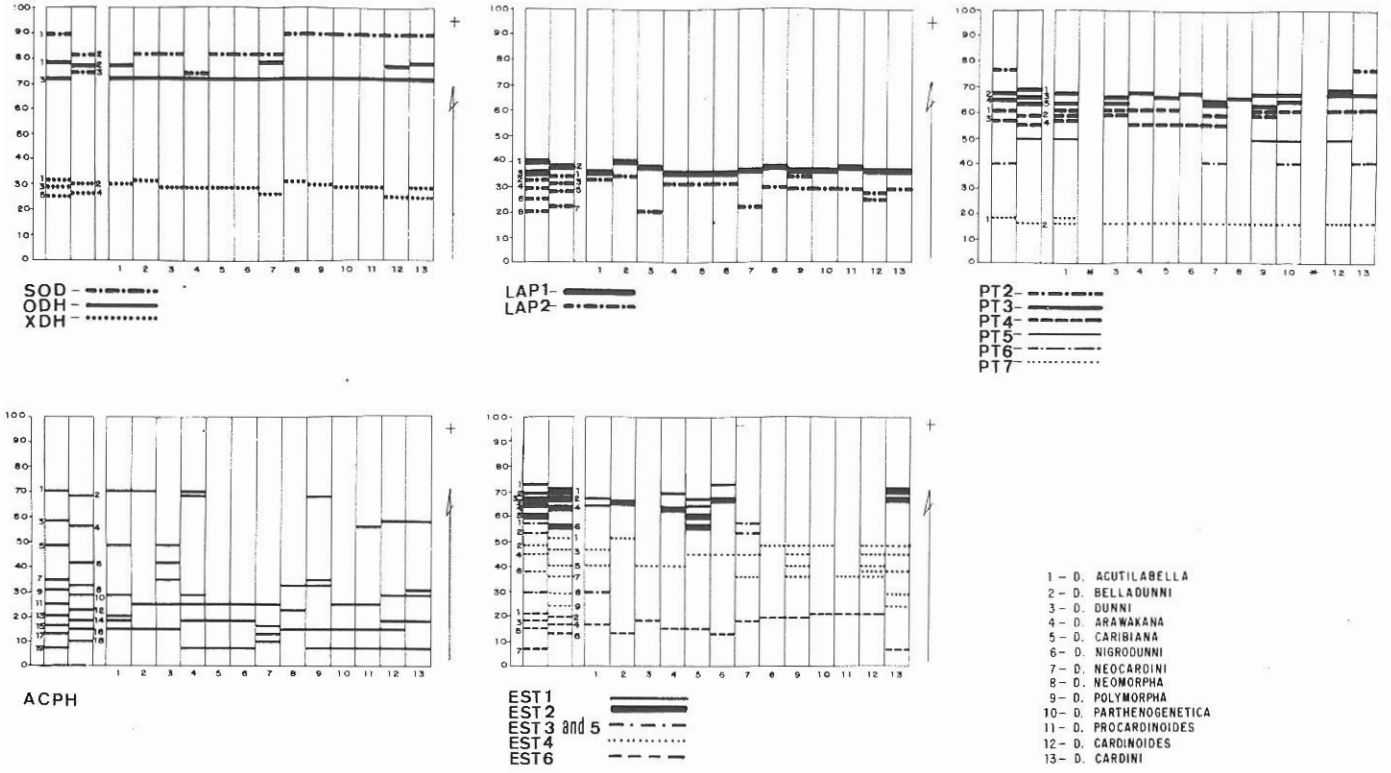

Figure 1 - Electrophoretic variants among 13 species of the *cardini* group. The total number of variants found for the entire group for each system studied is given in the first two colums.

*Species which were not analyzed for total protein.

Table II - Genetic similarity among 13 species of the *cardini* group.

Species	*acut.*	*bell.*	*dun.*	*araw.*	*carib.*	*nigr.*	*neoc.*	*neom.*	*poly.*	*part.*	*proc.*	*cards.*	*card.*
acutilabella	–	4/25	8/36	10/34	8/36	6/35	5/42	3/35	10/39	6/36	2/27	9/41	6/45
		.160	.222	.294	.222	.171	.119	.086	.256	.167	.074	.220	.134
belladunni		–	3/20	4/22	3/22	4/19	3/22	3/18	3/25	3/17	3/19	2/27	1/30
			.150	.182	.136	.211	.136	.167	.120	.176	.158	.074	.033
dunni			–	6/30	6/29	4/28	6/32	5/23	7/31	6/27	5/18	6/34	4/37
				.200	.207	.143	.188	.217	.226	.222	.278	.176	.108
arawakana				–	12/25	11/23	7/32	2/29	7/34	7/28	4/22	9/33	8/36
					.480	.478	.219	.069	.206	.250	.182	.272	.222
caribiana					–	11/22	6/32	3/27	5/36	6/28	4/21	6/35	7/35
						.500	.188	.111	.139	.214	.190	.171	.200
nigrodunni						–	7/30	2/24	5/33	6/25	4/19	5/33	8/31
							.233	.084	.151	.240	.211	.152	.258
neocardini							–	2/31	7/37	6/31	3/23	5/39	6/40
								.065	.189	.194	.130	.128	.150
neomorpha								–	7/28	5/23	4/17	5/30	4/33
									.250	.217	.235	.167	.121
polymorpha									–	11/28	6/22	11/31	10/38
										.393	.272	.355	.263
parthenogenetica										–	8/13	11/27	11/30
											.615	.407	.367
procardinoides											–	6/23	5/25
												.261	.200
cardinoides												–	14/34
													.412
cardini													–

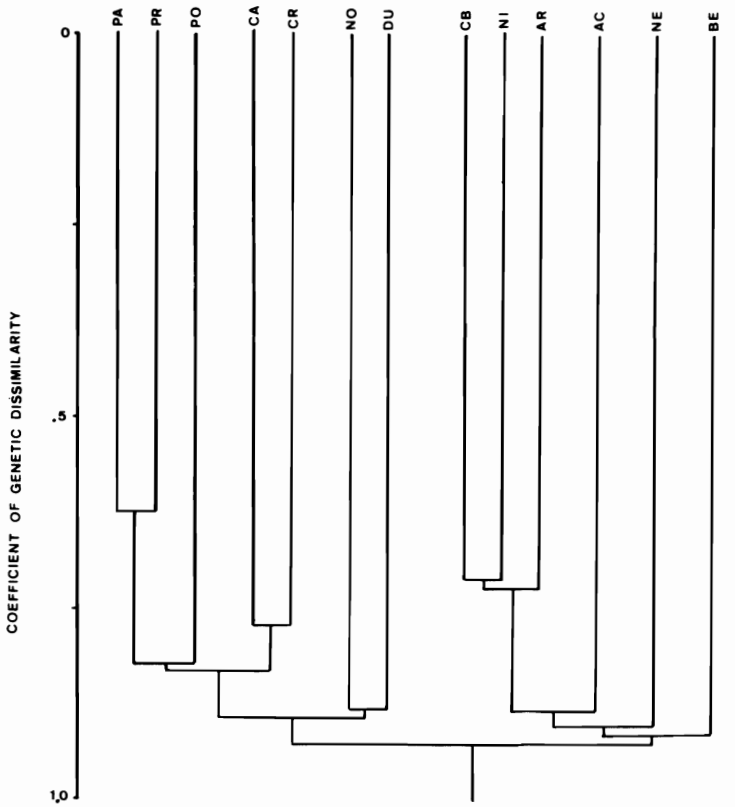

Figure 2 - Dendrogram showing the probable phylogenetic relationships among 13 species in the *cardini* group. (Abbreviations as in Table I).

Great genetic similarity was also found between *D. arawakana, D. caribiana* and *D. nigrodunni,* having about 50% coinciding alleles. According to Sneath and Sokal (1973), the I value (similarity index) should not be used in Numerical Taxonomy. Thus, we calculated the d values (euclidean distance) and constructed the dendrogram or phenogram (Figure 2) based on the unweighted pair-group method using arithmetic means (UPGMA) (Sneath and Sokal, 1973). The dendrogram indicates two main groupings of species: one consisting of *D. parthenogenetica, D. procardinoides, D. polymorpha, D. cardinoides, D. cardini, D. neomorpha,* and *D. dunni,* and the other consisting

almost entirely of insular species, plus *D. neocardini.* The presence of *D. acutilabella* and *D. neocardini* in this block is not surprising if we consider that these are the only two species in the *cardini* subgroup which hybridize with species of the *dunni* subgroup. On the basis of cytogenetic studies, *D. acutilabella,* or its ancestor, was considered to be the species that gave origin to this subgroup of insular species (Heed and Russel, 1971).

DISCUSSION

We found a mean genetic similarity of 21% in the *cardini* group. These values are comparable to those obtained by Hubby and Throckmorton (1968) for non-cryptic species pairs of *Drosophila* (18%). As reported by Selander and Johnson (1973), a wide range of values is expected when species are compared, because the differences reflect both the amount of genetic modification that accompanied the speciation process and that which accumulated after speciation occurred. A comparative study of species in the *mesophragmatica* group (Nair *et al.,* 1971) showed two types of situations: 1) different populations in the same species showing great genetic divergence without being reproductively isolated; 2) species already showing reproductive barriers without having diverged genetically. Ayala *et al.* (1975), in a study on the *willistoni* group of *Drosophila,* concluded that a large part of genetic divergence occurs during the first speciation stage, when allopatric populations have become sufficiently differentiated, but not yet reproductively isolated.

The amount of genetic differentiation shown by the species of the *cardini* group was highly variable; however, the values obtained (21%) were, on the average, much lower than those obtained for other *Drosophila* species (Nair *et al.,* 1971; Lakovaara *et al.,* 1972; Johnson *et al.,* 1975). Several investigators (Kanapi and Wheeler, 1970; Nair *et al.,* 1971; Yang *et al.,* 1972) have found agreement between the levels of interspecies relationships established by cytologic and genetic studies. However, Rockwood *et al.* (1971) found wide variation (26% to 80%) in genetic divergence when they compared pairs of homosequential *Drosophila* species from Hawaii, although homosequential species are on the average more genetically similar (45.3%) than pairs of species selected at random (34.4%).

Returning now to the *cardini* group, the seven species in the *dunni* subgroup represent two groupings of species having identical gene arrangements (Heed and Russel, 1971): *dunni, belladunni* and *similis,* forming one

cluster, and *arawakana, caribiana, antillea* and *nigrodunni* representing the other. Our isozyme data confirm the great similarity existing among *arawakana, caribiana, antillea* and *nigrodunni,* which coincide in about 50% of their alleles. However, the coefficient of similarity between *belladunni* and *dunni* is only 15%.

Johnson *et al.* (1975) consider values of genetic similarity among species to have little phylogenetic significance, unless supplemented with other data defining the types of foundation and speciation events that occurred during the evolution of a group of species. Within the hypothesis of the selective neutrality of protein variants, the differences observed in our study could be excellent indicators of the time each species took to diverge, but not of their real genetic divergence, which must be based on traits which have been shown to be significant for the adaptation of each species to its particular niche.

RESUMO

Padrões eletroforéticos de seis sistemas enzimáticos de adultos e de proteínas totais de pupas foram examinados para comparar treze espécies do grupo *cardini* de *Drosophila.* Comparações entre as espécies duas a duas mostram um grau de similaridade genética de aproximadamente 0.50 entre as espécies do complexo *arawakana-caribiana-nigrodunni.*

D. antillea, que pertence a este complexo, só foi analisada quanto à esterase, mostrando um padrão extremamente semelhante às outras. Da mesma forma, a tríade formada por *D. cardinoides, D. procardinoides* e *D. parthenogenetica* apresenta um índice médio de similaridade de 0.43. Estes resultados confirmam o que foi encontrado por outros autores quanto a afinidade reprodutiva e citologia. Entretanto, *dunni* e *belladunni,* que pertencem a um complexo de três espécies insulares muito semelhantes do ponto de vista citológico, apresentaram apenas 0.15 de similaridade.

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