

GENETIC VARIABILITY AND DISEQUILIBRIUM IN THE MAJOR ESTERASE LOCUS IN FOUR SPECIES OF THE *CARDINI* GROUP OF *DROSOPHILA*

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ABSTRACT

Samples of natural populations of *Drosophila cardini*, *cardinoides*, *neocardini* *itambacuriensis* and *polymorpha* showed significant disequilibrium in the esterase *est* 4 locus with respect to the Hardy-Weinberg rule. However, analysis of F₁ samples from wild inseminated females collected from two sites showed equilibrium frequencies. This fact suggests that different selection pressures could be operating from egg to adult and during the adult stage. The allele *est* 4^{1.0} is the most frequent in all collection sites of *D. polymorpha* and in two sites for *D. cardini*. The most frequent allele in the *cardinoides* population is one with the highest electrophoretic mobility (*est* 4^{1.1}). Populations of *neocardini* show a different major allele in each locality so far studied, *est* 4^{0.80} and the null allele (*est* 4⁰).

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INTRODUCTION

The *cardini* group of *Drosophila* contains 16 species, eight restricted to the Greater and Lesser Antilles, and eight to continental tropical and subtropical America. W.B. Heed has made the largest contribution to our knowledge of the genetics, reproductive isolation, color polymorphism, cytology, and phylogeny of this group (Heed, 1962; Heed and Krishnamurty, 1959; Heed and Blake, 1963; Heed and Russel, 1971). This species group has the greatest degree of color polymorphism among neo-tropical *Drosophila*.

The studies of Da Cunha (1949, 1955) on *polymorpha* and *neocardini*, Stalker (1953) and Heed (1962) on *acutilabella*, Heed and Blake (1963), and Martinez and Cordeiro (1970) on *polymorpha* have provided some information on the genetic control of this polymorphism although our understanding of it is quite incomplete.

We have carried out isoenzyme analysis of the esterases of this group to learn more about their population genetics, and to eventually correlate color and enzyme polymorphism.

MATERIAL AND METHODS

The flies were collected in southern Brazil in native woods by sweeping a net over banana-orange (1:1) bait fermented with baker's yeast. In Ponta do Caximbo, Porto Alegre, collections were made over naturally decaying fruits of *Aleurites molucana* (Euphorbiaceae). *Drosophila polymorpha* samples were obtained from Eldorado (Guaíba County), Restinga (Porto Alegre County), Iguaçu National Park, State of Paraná, Morro do Ferro, and Control wood in the region of high radiation background in Poços de Caldas, State of Minas Gerais. *Drosophila cardini* were collected in Eldorado, Restinga, and Ponta do Caximbo, and *Drosophila neocardini itambacuriensis* (Da Cunha, 1955) in Restinga and Ponta do Caximbo.

The captured wild flies and the F₁ generation from inseminated wild females were aged 8 days and stored frozen at -25° C. Gels of hydrolyzed starch (12%) or polyacrylamide (5 or 7%) were used in horizontal plates, with Poulik's (1957) discontinuous buffer system. A 10 V/cm electric field

was applied across the gels for 3 to 4 hours until the migrating front was 10 cm from the sample slot line. The gels were incubated at 38° C for 20 minutes in 0.1 M phosphate buffer and then in a mixture containing: 1% of alpha - and 1% beta-naphthylacetate and 1% fast blue BB salt until the major esterase bands appeared.

RESULTS

Most of the isozymes of the *est* 4 locus in *D. polymorpha* are shown in Figure 1. We observed the same pattern in the four species studied. The relative mobility was used when comparing different gels. The *est* 4^{1.0} was used as the reference marker for the alleles. Nine alleles were detected in *Drosophila polymorpha* (Table I). It was possible to distinguish 12 electrophoretic alleles in *Drosophila cardini* with relative mobilities ranging from 0.25 to 1.1 (Table II). Four alleles slower than those present in *polymorpha* were detected but no null allele was observed in the 255 individuals analyzed. *Drosophila n. itambacuriensis* showed 11 alleles (Table II). This locus is less variable in *D. cardinoides*. Heterozygosity was calculated according to Lewontin and Hubby (1966), and also by the formula $h = 1 - \sum x_i^2$ (Nei, 1975), where x_i is the frequency of the i^{th} allele. The same result was obtained with both calculations and these data are included in Tables I and II. The most common allele in the six samples of *polymorpha* is the *est* 4^{1.0}. Samples of *cardini* collected in Eldorado and Restinga in February and March '71 also have *est* 4^{1.0} as their major allele. However, in the Caximbo sample of April '74 *est* 4^{0.95} is the most frequent allele. In *D. n. itambacuriensis* the most frequent alleles are *est* 4^{0.80} in the Restinga sample of March '73 and *est* 4⁰ null allele in the Caximbo sample of April '76. The only sample of *D. cardinoides* (Caximbo, April '74) shows predominance of *est* 4^{1.1}.

To estimate the equilibrium frequency of zygotic proportions we reduced the data to three classes: 1) the homozygotes for the most frequent allele, 2) the pooled other homozygotes, and 3) the pooled heterozygotes. This was done because several allele frequencies were so low that some zygotic combinations did not appear. Except for F₁ samples from Eldorado and Tainhas and the Iguaçú sample of *D. polymorpha*, all other samples

Table I - Frequencies of the alleles of *est 4* and heterozygosity in several populations of *Drosophila polymorpha* from South Brazil.

Localities	Collecting Dates	Genomes Analyzed	<i>est 4</i>							Heterozygosity		
			<i>est 4</i> ^{1.1}	<i>est 4</i> ^{1.05}	<i>est 4</i> ^{1.0}	<i>est 4</i> ^{0.95}	<i>est 4</i> ^{0.9}	<i>est 4</i> ^{0.85}	<i>est 4</i> ^{0.8}			
Eldorado	2/1971	382	.005	.031	.757	.146	.037	.018	.003	.003	—	.403
Eldorado *+	6/1973	256	.008	.063	.656	.191	.039	.031	.012	—	—	.526
Eldorado	6/1973	234	.026	.085	.645	.180	.026	.038	—	—	—	.541
Restinga	3/1971	310	.003	.045	.765	.085	.061	.019	.006	.003	.013	.401
Tainhas	3/1972	98	.020	.031	.776	.122	—	.031	—	—	.020	.380
Tainhas *+	4/1973	164	.006	.067	.659	.183	.067	.012	.006	—	—	.523
Iguaçu *	3/1972	72	.014	.042	.805	.111	—	.028	—	—	—	.337
Poços de Caldas	1/1972	360	.005	.016	.882	.077	.005	.011	.002	—	—	.216
TOTAL		1876										

+ Data from *F₁* samples

* Samples in equilibrium according to Hardy-Weinberg rule.

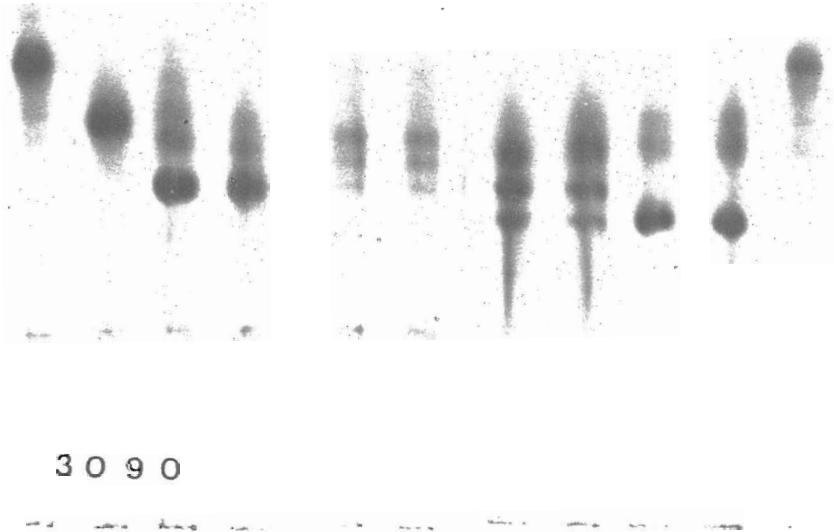


Figure 1 - Phenotypes of the est 4 loci in *D. polymorpha*. From left to right:
est 4^{1.1} / est 4^{1.1}; est 4^{1.0} / est 4^{1.0}; est 4^{.85} / est 4^{.85}; est 4^{.85} / est 4^{.85};
est 4^{.95} / est 4^{.85}; est 4^{.95} / est 4^{.85}; est 4^{.88} / est 4^{.75}; est 4^{.88} / est 4^{.75};
est 4^{.75} / est 4^{.75}; est 4^{.75} / est 4^{.75}; est 4^{1.1} / est 4^{1.1}.
Electrophoresis was carried out in 7% polyacrylamide gels, and esterase activity was detected as described in the Methods section.

Table II - Frequencies of the alleles of *est 4* and heterozygosity in populations of *D. cardini*, *D. itambacuriensis* and *D. cardinoides*.

Species	Localities	Dates	Collecting Genomes													
			Analyzed	<i>est 4^{1.1}</i>	<i>est 4^{1.0}</i>	<i>est 4^{0.9}</i>	<i>est 4^{0.8}</i>	<i>est 4^{0.7}</i>	<i>est 4^{0.6}</i>	<i>est 4^{0.5}</i>	<i>est 4^{0.4}</i>	<i>est 4^{0.3}</i>	<i>est 4^{0.2}</i>	<i>est 4^{0.1}</i>	Heterozygosity	
<i>D. cardini</i>	Eldorado	2/1971	130	.146	.338	.231	.031	.015	.023	.139	—	.031	.046	—	—	.787
	Restinga	3/1971	264	.004	.299	.152	.144	.030	.049	—	.087	.178	.057	—	—	.821
	Ponta do Caximbo	4/1974	116	—	.009	.207	.155	.112	—	.086	.069	.146	.069	.095	.052	.870
	TOTAL		510													
<i>D. neocardini</i>	Restinga	3/1971	78	—	.051	.090	.180	.051	.269	.064	.141	.051	.103	—	—	.845
	Ponta do Caximbo	4/1974	128	—	.016	—	—	.055	.109	.086	.148	—	.110	.023	—	.738
	TOTAL		206													
<i>D. cardinoides</i>	Ponta do Caximbo	4/1974	284	.644	.187	.074	.039	.007	.018	—	—	.003	—	—	.023	.542

of this species and the other three are not in agreement with the equilibrium frequencies predicted by the Hardy-Weinberg rule. There is an excess of homozygotes in the other samples of *D. polymorpha*, *D. cardinoides* and *D. n. itambacuriensis* from Caximbo. However, the sample of *D. n. itambacuriensis* from Restinga and the three samples of *cardini* show only an excess of one of the homozygote classes but also an excess of heterozygotes.

DISCUSSION

Drosophila polymorpha populations consistently showed a distinctly higher frequency for *est* 4^{1.0} than for any other allele. The other two species studied in more than one sample showed a different allele at the highest frequency for each collecting site. These interspecific variations of the most common allele are not the rule in *Drosophila* species, and different species usually do not share the same allele as the major one. We do not know the causes of these variations.

It is interesting to note that the naturally decaying fruits of *Aleurites molucana* at Caximbo attracted exclusively *D. cardini*, *D. cardinoides*, *D. neocardini*, some *polymorpha* and a few *simulans* individuals. A trap of banana and orange fermented with baker's yeast, placed at 20 meters from the *Aleurites* fermenting fruits, exhibited a varied fauna with 12 to 15 commonly occurring *Drosophila* species and only a few *Cardini* group individuals. The Caximbo results appear to differ from the others in the three first species. Unfortunately we have not analyzed other samples from this location which seems to be quite specialized for the *cardini* group of *Drosophila*.

If natural sites were more completely analyzed, the microgeographic (microecologic) structure of gene distribution might demonstrate that the prevalence of one allele is a niche character. The excess of homozygotes in populations of *D. polymorpha* may be explained by the Wahlund's principle, if a pooling of subdivided populations is occurring. The pooling of low frequency alleles and the grouping together of their heterozygotes, as we did to estimate the equilibrium frequency of zygotic proportions, might also be inappropriate.

Another possibility is that heterozygotes have an advantage (heterotic selection) from the egg stage to the pupa stage, while the adult homozygotes are better adjusted to several specialized niches due to diversifying selection. Da Cunha (1949), studying color polymorphism, also found an excess of homozygotes in samples of adults of *D. polymorpha* from nature, and excellent agreement with the Hardy-Weinberg rule in laboratory cultures, when the adults were not submitted to selection pressure. This is suggested in our data by the contrasting results of the wild fly samples (adult samples) and the F_1 sample of Eldorado and Tainhas (Table I). However, the F_1 sample of *D. polymorpha* from Poços de Caldas showed an excess of homozygotes also, which might be explained by very low larval competition. Another problem is the predominance of the null allele (*est* 4°) since a great portion of heterozygotes would bear it and would be scored as homozygotes. Only an F_1 analysis could clarify this point:

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