ISOZYME FREQUENCY PATTERNS IN *DROSOPHILA PAVANI* ASSOCIATED WITH GEOGRAPHICAL AND SEASONAL VARIABLES[1]

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ABSTRACT

Fourteen population samples of Drosophila pavani were obtained from a number of localities in Chile. The populations sampled were dispersed over 7 degrees of latitude and 1800 meters of elevation, and were drawn at three different times. Sixteen electrophoretic loci were assayed for each population; eight of the loci were analyzed statistically for geographic variation; the other eight were essentially monomorphic. For all eight variable loci, variation in allelic frequencies among populations was highly significant. In all cases, a significant portion of the variation among populations was associated with variation in gross environmental variables (latitude, elevation, month of collection). The implications of the evidence were discussed, and the authors concluded that there was suggestive evidence for selection.

AN impressive body of data has accumulated in the last few years which indicates that large quantities of electrophoretically detectable genetic variation exist in natural populations of most species. Because of the continuing controversy over the adaptive significance of this variation, a number of investigators have been led to compare the allelic frequencies of different populations (e.g., JOHNSON *et al.* 1969; SELANDER, YANG and HUNT 1969; PRAKASH, LEWONTIN and HUBBY 1969). While there have been various opinions expressed as to the proper interpretation of geographic differences, or lack thereof, it is generally conceded that when patterns of genetic variation are associated with corresponding patterns of environmental variation, there is at least a strong suggestion that the genetic variation in question is adaptive. The objective of this study is to investigate the patterns of isozyme variation among populations of *Drosophila pavani*, in an attempt to determine whether there is any evidence for selection at isozyme loci. Specifically, we wish to answer three questions: (1) Are there differences in allelic frequencies among populations? (2) Are such differences associated with geographical and environmental variables in linear fashion? (3) Are there residual differences among populations, above and beyond those linearly associated with the environment?

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MATERIALS AND METHODS

Collections: Drosophila pavani is a neotropical species which belongs to the *mesophragmatica* group of the subgenus Drosophila. This species has a limited geographic range in central Chile, occurring between latitudes 27° S and 40° S on the western slopes of the Andes. It also extends eastward of the Andes into Argentina, where it overlaps the distributional area of the sibling species *D. gaucha* (BRNCIC 1970). The present study is limited to populations inhabiting the relatively narrow strip along the Chilean coastal range. The area sampled covers 7° of latitude and 1800 m of elevation, and should provide considerable opportunity for environmental variation. Because of the extensive area covered, it was not possible to obtain all population samples simultaneously, and it became necessary to make three separate sets of collections.

(1) January, 1971: This set represents an elevational transect at about the latitude of Santiago. The set includes collections 1–5 in Table 1.

(2) March, 1971: This set represents both elevational and latitudinal transects, and includes collections 6–10 in Table 1.

(3) April, 1971: This set is primarly a latitudinal transect at lower elevations, and includes collections 11–14 in Table 1.

Only a single location is sampled twice. This is San José de Maipo (3,9), which was sampled in both January and March. It can be seen from Table 1 that any effects of latitude and/or elevation are overlain by any seasonal differences which might exist.

Wild-caught adults were placed in vials with food, were allowed to oviposit, and were then shipped to Austin. Because of heavy mortality among the parents during shipment (but subsequent to oviposition), it was felt that possible biases could be avoided by using data from F_1 progeny only. The data presented below were obtained entirely from F_1 flies.

Electrophoretic assay: As many isozyme loci as possible were assayed on each individual, but it was not possible to assay all system on the same set of flies. To simplify presentation, the loci are arbitrarily divided into three sets: (A), Esterase-2 (Est-2), Esterase-6 (Est-6), Esterase-C (Est-C), and Isocitrate dehydrogenase (IDH); (B), Glucose-6-Phosphate dehydrogenase (G6PD), Phosphoglucomutase (PGM), Phosphoglucoisomerase (PGI), and Alkaline phosphotase (APH); (C), Aldehyde oxidases-1 and -2 (AO-1, AO-2), Hexokinase-1 (HK-1), α-Glycerol-phosphate dehydrogenase (αGPD), Glutamate oxaloacetate transaminase (GOT), 6-Phospho-gluconate dehydrogenase (6PGD), Acid phosphatase (ACPH), and Leucine amino peptidase (LAP). For set A, 711 flies were assayed for all four loci. For set B, large numbers of flies were assayed for

TABLE 1

Sample locations, with elevation, latitude and month of collection

Location	Month of collection	Latitude	Elevation
(1) El Tabo	January	33°28′	25m
(2) Bellavista	January	33°34′	600m
(3) San José de Maipo	January	33°39′	967m
(4) Melipilla	January	33°41′	164m
(5) Volcan	January	33°50′	1800m
(6) La Serena	March	29°55′	60m
(7) Vicuña	March	30°02′	650m
(8) Leyda	March	33°37′	100m
(9) San José de Maipo	March	33°39′	967m
(10) Copiapo	April	27°34′	381m
(11) Vallenar	April	28°44′	384m
(13) Rancagua	April	34°10′	500m
(13) San Fernando	April	34°35′	334m
(14) Santa Cruz	April	34°38′	164m

each locus, but the loci were assayed in several overlapping sets. It is perhaps simplest to treat these four loci as though each had been sampled on a separate set of flies. The loci of set C are represented by spotty samples or are essentially monomorphic for all populations, and are consequently not discussed further. Techniques of electrophoretic assay for all of these loci are given in KOJIMA, GILLESPIE and TOBARI (1970).

Statistical treatment: The statistical methodology employed for this study is thoroughly described in a companion paper by SMOUSE and KOJIMA (1972), and the reader is referred to that paper for details. Briefly, we assume that the gametic genotypes encountered within a given population have arisen from a multinominal distribution, with each genotype having an expected frequency appropriate for that population. The three questions of interest may be answered (in a statistical sense) by comparing the closeness of the fit provided by each of the following three hypotheses:

$$H_0: \quad P_i = P \qquad i = 1, \ldots, I$$
$$H_1: \quad P_i = \beta_0 Z_{0i} + \beta_1 Z_{1i} + \beta_2 Z_{2i} + \beta_3 Z_{3i} \qquad i = 1, \ldots, I$$
$$H_2: \quad \text{Not all } P_i \text{ the same.}$$

The parameter P_i is the expected frequency of the allele A_1 of a two allele locus in population (i); Z_0 is a dummy regression variable; Z_1 is the elevation in meters; Z_2 is (latitude-27°); Z_3 is a crude index of seasonal climatic differences($Z_{3i} = 0$ for January collections, $= 2$ for March collections, $= 3$ for April collections). This last convention is arbitrarily linear, and is employed in ignorance of how seasons actually affect gene frequencies. Three-allele loci are treated in analogous fashion.

The analysis consists of a set of likelihood ratio test criteria which compare the three hypotheses. By partitioning the hypothesis H_1 (as illustrated by SMOUSE and KOJIMA 1972), we have also obtained various partitions of the regression component, in order to gauge the relative contributions of the three environmental measures.

RESULTS AND CONCLUSIONS

The observed allelic frequencies for the loci of set A are listed for each population in Table 2, while those for set B are listed, along with sample sizes, in Table 3. The Est-2 locus exhibits a staining (S) allele and a non-staining (N) allele. The heterozygote is lightly stained, and is separately detectable. The Est-6, Est-C, and IDH loci have three alleles each, which are denoted F (fast), M (medium), and S (slow) for convenience. The allelic frequencies of Est-6, Est-C, and IDH vary considerably among populations, while those for Est-2 are much less variable. The APH locus exhibits three alleles (F, M, and S), and all three alleles vary considerably in frequency among populations. The G6PD, PGM, and PGI loci exhibit two alleles each in varying frequencies.

The analyses for two-allele loci are presented in Table 4. We present two alternative orders of fitting the repression variables: (elevation, latitude, and season), and (latitude, elevation, and season). We have not presented the other four possible orders, because we feel that season should be treated as modifying the basic pattern indicated by latitude and elevation.

The variation among populations is significant ($P < .01$) for all four loci. While some caution is in order, relative to exact probability levels (because of small expected numbers for some classes), it is nevertheless clear that differences among populations for allelic frequencies are appreciable. The portion of the variation among populations attributable to regression is also significant in all cases ($P < .01$). The joint contribution of latitude and elevation is significant for

TABLE 2

Allelic frequencies of fourteen population samples of Drosophila pavani *(loci of set A)*

Population code number	Haploid sample size	Esterase-2		Esterase-6			Esterase-C			IDH		
		Null (N)	Staining (S)	Fast (F)	Medium (M)	Slow (S)	Fast (F)	Medium (M)	Slow (S)	Fast (F)	Medium (M)	Slow (S)
(1)	84	.964	.036	.905	.083	.012	.012	.988	.000	.536	.179	.285
(2)	96	.969	.031	.573	.281	.146	.448	.385	.167	.167	.427	.406
(3)	100	.960	.040	.730	.270	.000	.520	.450	.030	.140	.740	.120
(4)	98	.990	.010	.551	.367	.082	.551	.439	.010	.316	.204	.480
(5)	94	.840	.160	.766	.192	.042	.574	.415	.011	.319	.543	.138
(6)	116	.888	.112	.517	.483	.000	.483	.457	.060	.405	.423	.172
(7)	104	.990	.010	.442	.558	.000	.163	.827	.010	.183	.788	.029
(8)	96	.969	.031	.854	.146	.000	.260	.698	.042	.667	.323	.010
(9)	96	.969	.031	.719	.229	.052	.271	.719	.010	.396	.490	.114
(10)	110	.918	.082	.736	.173	.091	.445	.482	.073	.409	.509	.082
(11)	104	.923	.077	.558	.183	.259	.519	.413	.068	.058	.856	.086
(12)	106	.934	.066	.453	.236	.311	.472	.481	.047	.179	.745	.076
(13)	110	1.000	.000	.527	.155	.318	.582	.354	.064	.118	.782	.100
(14)	108	1.000	.000	.491	.509	.000	.222	.778	.000	.176	.759	.065
Total/ Average	1422	.951	.049	.662	.281	.097	.400	.557	.013	.286	.564	.150

TABLE 3

Allelic frequencies and sample sizes (n) of fourteen population samples of Drosophila pavani *(loci of set B)*

Population code number	G6PD			PGM			PGI			APH			
	(n)	Fast (F)	Slow (S)	(n)	Fast (F)	Slow (S)	(n)	Fast (F)	Slow (S)	(n)	Fast (F)	Medium (M)	Slow (S)
1	(104)	.000	1.000	(104)	1.000	.000	(104)	1.000	.000	(84)	.488	.429	.083
2	(40)	.175	.825	(40)	1.000	.000	(40)	1.000	.000	(98)	.490	.459	.051
3	(98)	.051	.949	(98)	1.000	.000	(98)	1.000	.000	(96)	.406	.333	.261
4	(102)	.020	.980	(102)	1.000	.000	(102)	1.000	.000	(94)	.606	.245	.149
5	(92)	.174	.826	(92)	.935	.065	(92)	1.000	.000	(94)	.521	.319	.160
6	(108)	.278	.722	(108)	1.000	.000	(100)	.670	.330	(74)	.824	.041	.135
7	(104)	.433	.567	(104)	.808	.192	(104)	.702	.298	(74)	.730	.230	.040
8	(106)	.264	.736	(106)	.783	.217	(98)	.612	.338	(96)	.438	.469	.093
9	(104)	.210	.760	(104)	.837	.163	(70)	.571	.429	(94)	.415	.489	.096
10	(108)	.083	.917	(108)	.713	.287	(108)	.852	.148	(44)	.886	.023	.091
11	(80)	.000	1.000	(80)	.637	.363	(46)	.522	.478	(60)	1.000	.000	.000
12	(108)	.306	.694	(108)	.954	.046	(108)	.694	.306	(80)	.388	.538	.074
13	(108)	.287	.713	(108)	.954	.046	(108)	.750	.250	(58)	.603	.397	.000
14	(108)	.250	.750	(108)	.833	.167	(108)	.815	.185	(122)	.426	.172	.402
Total/ Average	(1370)	.188	.812	(1370)	.888	.112	(1286)	.806	.194	(1168)	.554	.313	.133

TABLE 4

χ^2 analysis of population variation for two-allele loci

Source of variation	Degrees of freedom	Asymptotic χ^2			
		Est-2	G6PD	PGM	PGI
Among populations	13	61.87	191.38	200.89	272.46
Regression	3	27.17	71.83	115.95	198.10
Z_1	1	7.74	.65	.48	21.53
$Z_2 \mid Z_1$	1	17.97	1.70	44.05	8.63
$Z_3 \mid Z_1,Z_2$	1	1.46	69.48	71.42	167.94
Z_2	1	8.54	1.57	44.16	11.97
$Z_1 \mid Z_2$	1	17.17	.78	.37	18.19
$Z_3 \mid Z_1,Z_2$	1	1.46	69.48	71.42	167.94
Lack of fit	10	34.70	119.55	84.94	74.36

Z_1 = Elevation
Z_2 = (Latitude —27°)
Z_3 = Seasonal index

$\chi^2_{1,\ .01} = 6.63$ $\chi^2_{10,\ .01} = 23.2$
$\chi^2_{3,\ .01} = 11.3$ $\chi^2_{13,\ .01} = 27.7$

TABLE 5

Comparison of observed allelic frequencies with those predicted under the full regression model (two-allele loci)

Population code number	Esterase-2 null allele		G6PD slow allele		PGM fast allele		PGI fast allele	
	Obs.	Expt.	Obs.	Expt.	Obs.	Expt.	Obs.	Expt.
1	.964	.977	1.000	.993	1.000	1.000†	1.000	1.000+
2	.969	.953	.825	.936	1.000	1.000†	1.000	1.000+
3	.960	.938	.949	.900	1.000	1.000†	1.000	1.000+
4	.990	.974	.980	.977	1.000	1.000†	1.000	1.000+
5	.840	.903	.826	.817	.935	.957	1.000	1.000+
6	.888	.941	.722	.862	1.000	.856	.670	.762
7	.990	.917	.567	.804	.808	.825	.702	.762
8	.969	.984	.736	.818	.783	.920	.612	.784
9	.969	.946	.760	.734	.837	.872	.571	.784
10	.918	.903	.917	.774	.713	.732	.852	.640
11	.923	.917	1.000	.761	.637	.753	.522	.647
12	.934	.977	.694	.690	.954	.843	.694	.679
13	1.000	.989	.713	.702	.954	.860	.750	.682
14	1.000	.997	.750	.717	.833	.871	.815	.682
β_0	.901371		1.006636		.934977		.961268	
β_1	—.000044		—.000097		—.000056		—.000001	
β_2	.011880		—.010947		.017921		.005993	
β_3	.004042		—.083158		—.063936		—.108223	

† The value of $\hat{P_i} = \hat{\beta_0}Z_{0i} + \ldots + \hat{\beta_3}Z_{3i}$ was greater than unity.

Est-2, PGM, and PGI, but not for G6PD. The relative contributions of these two variables depend upon the order of fitting. It is obvious, however, that elevation is more informative for PGI than is latitude, and that the reverse is true for PGM. Elevation and latitude seem to be jointly more effective in describing Est-2 frequencies than either variable alone would indicate. The most striking feature of Table 4, however, is the very large seasonal component of the variation for G6PD, PGM, and PGI. This component is not significant for Est-2.

The expected allelic frequencies (under the full regression model) and the estimated regression coefficients are presented for the two-allele loci in Table 5. There is a rough correspondence between observed and expected values, although the fit is far from perfect. The correspondence is closest for PGM and PGI, as would be predicted from the results of Table 4. The fit for G6PD is moderately good, while that for Est-2 is subtle at best.

The analyses for three-allele loci are presented in Table 6. In keeping with Table 4, two alternative orders of fitting are presented. The variation among populations is significant ($P < .01$) for all three-allele loci, as is the portion attributable to regression. The joint contribution of elevation and latitude is significant ($P < .01$) for Est-C, IDH, and APH, but not for Est-6. Elevation is the better single variable for Est-C and IDH, whereas latitude is more effective for APH. The seasonal component is significant for Est-6 and IDH, but not for Est-C and APH.

The expected allelic frequencies (under the full regression model) and the estimated regression coefficients are presented for three-allele loci in Table 7. The correspondence between expected and observed values is rather good for APH, and is adequate for IDH. Esterase-6 and Est-C are remarkable more for their lack of fit than for any pattern. While the regression components for both of these loci are highly significant, the description is poor in both cases.

TABLE 6

χ^2 analysis of population variation for three-allele loci

Source of variation	Degrees of freedom	Asymptotic χ^2			
		Est-6	Est-C	IDH	APH
Among populations	26	350.82	265.32	405.68	298.04
Regression	6	50.36	19.78	173.24	138.76
Z_1	2	4.80	13.96	15.26	8.76
$Z_2 \mid Z_1$	2	2.10	4.26	14.48	129.16
$Z_3 \mid Z_1,Z_2$	2	43.46	1.56	143.50	.84
Z_2	2	2.44	3.52	10.72	134.74
$Z_1 \mid Z_1,Z_2$	2	4.46	14.70	19.02	3.18
$Z_3 \mid Z_1,Z_2$	2	43.46	1.56	143.50	.84
Lack of fit	20	300.46	245.54	232.44	159.28

Z_1 = Elevation
Z_2 = (Latitude —27°)
Z_3 = Seasonal index

$\chi^2_{2,\ .01} = 9.21$ $\chi^2_{20,\ .01} = 37.6$

$\chi^2_{6,\ .01} = 16.18$ $\chi^2_{26,\ .01} = 45.6$

TABLE 7

Comparison of observed allelic frequencies with those predicted under the full regression model (three-allele loci)

Population code number	Esterase-6				Esterase-C				IDH				APH			
	Fast		Medium		Fast		Medium		Fast		Medium		Fast		Medium	
	Obs.	Expt.	Obs.	Expt.	Obs.	Expt.	Obs.	Expt.	Obs.	Expt.	Obs.	Expt.	Obs.	Expt.	Obs.	Expt.
1	.905	.677	.083	.284	.012	.314	.988	.631	.536	.427	.179	.325	.488	.533	.429	.330
2	.573	.692	.281	.264	.448	.386	.385	.570	.167	.358	.427	.371	.490	.503	.459	.362
3	.730	.701	.270	.251	.520	.432	.450	.531	.140	.314	.740	.458	.406	.482	.333	.384
4	.551	.680	.367	.278	.551	.331	.439	.618	.316	.409	.204	.269	.606	.512	.245	.348
5	.766	.722	.192	.221	.574	.537	.415	.443	.319	.214	.543	.655	.521	.435	.319	.434
6	.517	.611	.483	.314	.483	.362	.457	.578	.405	.341	.423	.478	.824	.791	.041	.128
7	.442	.626	.558	.294	.163	.436	.827	.516	.183	.271	.788	.618	.730	.758	.230	.162
8	.854	.593	.146	.287	.260	.346	.698	.609	.667	.312	.323	.508	.438	.515	.469	.329
9	.719	.616	.229	.258	.271	.455	.719	.516	.396	.210	.490	.713	.415	.478	.489	.372
10	.736	.589	.173	.324	.445	.428	.482	.514	.409	.267	.509	.668	.886	.949	.023	.010
11	.558	.583	.183	.316	.519	.422	.413	.525	.058	.259	.856	.675	1.000	.863	.000	.073
12	.453	.558	.236	.274	.472	.405	.481	.564	.179	.209	.745	.733	.388	.456	.538	.372
13	.527	.551	.155	.276	.582	.381	.354	.586	.118	.226	.782	.696	.603	.432	.397	.386
14	.491	.547	.509	.282	.222	.359	.778	.605	.176	.246	.759	.656	.426	.435	.172	.381
β_0	.710022		.329806		.349114		.571943		.471830		.192929		1.012960		—.019966	
β_1	.000027		—.000034		—.000126		—.000108		—.000118		.000235		—.000040		.000047	
β_2	—.005249		—.006982		—.005869		.009536		—.006540		.005647		—.074005		.059922	
β_3	—.042759		.003559		.011503		—.007549		—.052162		.127231		—.002048		—.006102	

It would be helpful to have an overview of the genetic variation among populations. SMOUSE and KOJIMA (1972) have pointed out that if loci are segregating independently within populations (linkage equilibrium), the multilocus analysis degenerates to the sum of the single-locus components. A more elaborate analysis is appropriate when linkage equilibrium does not obtain. Inasmuch as not all individuals were characterized for all loci, it was not possible to check for all interlocus correlations. In particular, we have virtually no information on interlocus correlations between the loci of sets A and B, and the informaiton on interlocus correlations within set B is very spotty.

An approximate overview may nevertheless be obtained by simply ignoring whatever linkage disequilibrium might exist within populations. Adding across loci (across columns of Tables 4 and 6) yields a total "Regression" component of 795.19 with 36 degrees of freedom. This constitutes only 38.9% of the "Among Populations" variation (2046.46), but is nevertheless enormously significant. There is obviously an association between genetic and environmental variation. The order of fitting latitude and elevation is largely irrelevant; latitude is clearly the more informative variable. The effect of elevation is nevertheless highly significant. The seasonal component is most impressive when one considers the crudeness of the seasonal index.

The "Lack of Fit" component is also highly significant (1251.27 with 120 degrees of freedom), indicating that the particular linear model employed is not adequate to accurately describe the genetic variation among populations. In view of the fact that latitude, elevation, and seasonal index are almost surely crude indicators of a host of correlated climatic and biotic variables, this is not at all surprising. Lacking a better environmental description, we shall content ourselves with demonstrating the general tendencies already indicated.

DISCUSSION

The results of this study are open to at least two very different interpretations. Under a selectionist interpretation, the association between genotypic and environmental variation is indicative that the genetic variation is adaptive. The residual lack of fit would most likely be interpreted as a failure to describe adequately the environments so crudely indexed by latitude, elevation, and seasonal index. Under a neutralist interpretation, the large lack of fit term would be attributed to random genetic drift at neutral loci. The population sizes are unknown, but some of them are probably not overly large. The genotype-environment associations might be attributed to the smoothing effects of migration among nearby populations, which populations might be only incidentally environmentally similar. There is, of course, no reason why both viewpoints might not be partially valid. There might well be selection, overlaid by random genetic drift and migrational smoothing. The data presented above are inherently circumstantial in nature, but several factors indicate to us that selection is probably the cause of at least a portion of the variation encountered among populations.

Environmentally similar populations are not necessarily close in a migrational

sense. Ideally, one should be able to contrast environmental and migrational distance, and then determine whether populations are more representative of their "geographical location" of their "environmental milieu". The comparison between environmental and migrational distance is a difficult proposition, however, without a far better understanding of both than is available for this study. The distribution of *Drosophila pavani* suggests that migration occurs up and down the valleys and north and south along the coast. It is not clear whether migration is more pronounced latitudinally or elevationally, but the fact that variation at some loci is more associated with latitude than elevation (PGM and APH), and some the reverse (PGI, Est-C, and IDH), does not seem compatible with random genetic drift, coupled with migrational smoothing. The large seasonal components of G6PD, PGM, PGI, Est-6, and IDH simply cannot be attributed to genetic drift and migration.

There is, of course, no guarantee that the loci observed are those under selection or that all of the effect observed is due to the loci themselves. Each of these loci is linked to others nearby, and it is likely that the effects observed are due, at least in part, to these linked loci. The possibility of an electrophoretic locus serving as a marker for an assemblage of loci is particularly important where inversions are present. Large selective effects of the inversion system might well override a smaller effect due to a single constituent locus.

There are three known inversion systems in *Drosophila pavani*, two on the fourth chromosome, and one on the second. It is not known which of the loci of this study reside on which chromosomes, except for APH. This locus is strongly associated with the inversion system on the right arm of chromosome IV (NAIR and BRNCIC 1971). It is possible that one or more of the other loci assayed in this study will ultimately be found associated with one or more inversion systems, but no information is available on this point at this time. While admitting that the patterns observed may well represent the effects of selection on blocks of genes, we see no necessity in assuming that the only detectable loci of these putative assemblages are neutral.

In conclusion, we submit that the association between genotypic and environmental variation in *Drosophila pavani* is quite suggestive of natural selection. We are inclined to ascribe at least a portion of this selection to the isozyme loci themselves.

Final note: The final drafts of this paper were prepared after the untimely death of Dr. KEN-ICHI KOJIMA. The respective responsibilities of the remaining authors were: SMOUSE—statistical analysis and manuscript preparation; YANG—electrophoretic analysis and interpretation; NAIR—preliminary electrophoretic and cytological analysis; BRNCIC—preliminary electrophoretic and cytological analysis and collection of population samples.

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