ISOZYME GENOTYPE-ENVIRONMENT ASSOCIATIONS IN NATURAL POPULATIONS OF THE HARVESTER ANT, *POGONOMYRMEX BADIUS*[1]

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ABSTRACT

Harvester ants (*P. badius*) were collected from 49 localities in the southeastern U.S. Amylase and naphthylamidase activities were assayed by gel electrophoresis, and the frequencies of alleles controlling electrophoretic variation were calculated. Soil samples were analyzed and plotted against allelic frequencies. Climatographic and genetic data were analyzed by the method of principal components. Statistically significant correlations were found to occur between the patterns of genetic variation, and between the genetic and environmental patterns. These correlations are consistent with a selective basis for the maintenance of isozyme polymorphisms.

HYPOTHESES of selection and neutrality for the maintenance of isozyme polymorphisms in natural populations are the basis of a current controversy in population genetics. Electrophoretically resolved isozyme polymorphisms exist at levels high enough (30% of all loci) to produce loads theoretically inconsistent with reproductive potentials (HALDANE 1957; KIMURA 1968; JOHNSON *et al.* 1966; LEWONTIN and HUBBY 1966; HARRIS 1966; STONE *et al.* 1968). For this reason, neutralists hypothesize on the basis of mathematical models that isozyme polymorphisms are the result of selectively neutral or nearly neutral mutations. These polymorphisms are established and maintained by isolation, migration and drift—non-Darwinian evolution (KIMURA 1968; KING and JUKES 1969; ARMHEIM and TAYLOR 1969; MARUYAMA 1970; ROBERTSON 1970). Those who favor selection maintain that the polymorphisms are controlled by selective processes (MAYNARD SMITH 1968, 1970; CLARKE 1970; RICHMOND 1970); i.e., heterosis (WILLS and NICHOLS 1971, 1972), density-dependent selection (SEMEONOFF and ROBERTSON 1968; POLIVANOV 1969), multiple niche polymorphism (POWELL

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1971) and frequency-dependent selection (KOJIMA 1971). Many observed isozyme polymorphisms show constant frequencies (PRAKASH, LEWONTIN and HUBBY 1969; SELANDER, HUNT and YOUNG 1969; AYALA *et al.* 1972) or clinal variation (KOEHN 1969) over wide geographic ranges. These polymorphisms could be stages of the fixation of a neutral mutation in a species (KIMURA and OHTA 1971), but demonstrations of isozyme genotype-environment relationships strengthen the argument for natural selection of isozyme polymorphisms (JOHNSON *et al.* 1969; MERRIT 1972; WILLS 1973; ROCKWOOD-SLUSS, JOHNSTON and HEED 1973).

If environmental factors which vary over the range of a species determine the relative fitness of genotypes, correlations may be expected to exist between these environmental factors and the genetic constitution of the organism. Such a correlation was found in a study of harvester ant (*Pogonomyrmex barbatus*) populations in Texas (JOHNSON *et al.* 1969) in which the observed genetic factors were polymorphic isozyme loci and the environmental factors included meteorological and botanical data. The correlations suggested a causal effect.

To further analyze the possible influence of natural selection, the following investigation was initiated to study allelic isozymes throughout the range of a different harvester ant (*P. badius*). Populations of this species, like those of *P. barbatus*, are stable and maintained in high densities throughout the year. Although the worker ants may forage far from the nest, the winged reproductive forms are the only source of genetically effective migration. The capacity for movement from unfavorable nest habitat is limited to the exodus and relocation of the queen and some progeny. This movement is infrequent and successful only over short distances, usually within 16 feet of the original location (*Fundamental Nuclear Energy Research*, 1969). Nests, containing a queen and numerous progeny, are long-lived in comparison with the natural life span of Drosophila and most other insects previously studied for isozyme variations.

MATERIALS AND METHODS

Harvester ants (*P. badius*) were collected (Table 1) in Alabama, Florida, Georgia, North Carolina and South Carolina. These states and the coastal areas of Louisiana and Mississippi form the known range of the species. Collections were made in a three-year period (1969–1971), and some localities were sampled more than once. Since nest positions are not constant, the same nests were not necessarily sampled on subsequent collections. Harvester ants occur in the greatest abundance in open, well-drained areas, such as abandoned fields, pastures, unkept lawns and road right-of-ways. These areas constituted the localities sampled. No regular distances or patterns were established in making the collections.

Individuals of the worker caste, nonreproductive diploid females, were collected from each nest sampled. The nests were disturbed with a spade, which usually caused large numbers of workers to come to the surface. As they emerged, the worker ants were scraped into a labelled vial. This procedure eliminated the possibility of collecting a passing ant from another nest. During winter in the northern regions and summer in the southern regions, nests were often excavated with a pick and shovel. About fifteen nests per locality were sampled.

Subcastes of the worker ants vary in overall size and relative head size (COLE 1968). The smallest (minor and median) workers were collected for analysis. These individuals are the most common and the easiest to collect. Thus, they provide the simplest means of obtaining a uniform sampling of the population. Comparisons of the small workers with the larger members

of the caste, the immature forms, the mature males and the mature queens would be desirable because the genotypic proportions possibly vary among them. The additional time required for collection, identification and analysis, however, precluded investigation of this type.

After the ants were collected, the labelled vials were packed in an insulated chest containing ice. At the laboratory, the ants were transferred to and sealed in very small vials, and frozen until analyzed. Enzyme phenotypes could be determined as long as two years after freezing.

The variation in two enzymes, amylase (AMY) and a naphthylamidase ("leucine aminopeptidase"—LAP), was investigated. Five individuals per nest were arbitrarily selected from those collected and analyzed for each enzyme. This sample of ten alleles provided the most accurate estimate of gene frequencies within the limits of available laboratory resources. Techniques for homogenate preparation and electrophoresis were those used by JOHNSON *et al.* (1969). The thorax was excised and homogenized. Polyacrylamide gels (seven percent) were used for AMY analysis and starch gels for LAP analysis. POULIK's (1957) buffer system was used for both. After electrophoresis, the gels were sliced horizontally and one or more slices were stained for enzyme activity. AMY was stained by incubating the gel for three hours at 37° in a two percent solution of amylopectin, followed by several rinses with water and the addition of a one percent iodine solution (cf. DOANE 1967). LAP was stained with L-leucyl-β-naphthylamide as the substrate and Black K salt as the dye coupler in a trismaleate buffer (cf. BECKMAN and JOHNSON 1964).

To compare the AMY variants on the basis of a characteristic other than electrophoretic mobility, some gels were incubated in water at high temperatures before staining. This treatment consisted of immersion in water at 50°, 60°, 70° or 80° for two minutes. The relative staining intensities within treated gels, within control gels (incubated in water at room temperature), and between treated and control gels were ascertained by visual examination.

Genetic variability patterns and environmental variability patterns were determined and analyzed by the principal component method (KENDALL 1957; JOHNSON *et al.* 1969). Environmental data were obtained from U.S. Climatographic Records and *A Forest Atlas of the South.* Soil samples were analyzed for pH, organic content, chemical elements and nitrate by the Soil Testing Division of the North Carolina Department of Agriculture. These determinations were made in accordance with the *Official Methods of Analysis of the Association of Official Agricultural Chemists.*

RESULTS AND DISCUSSION

Amylase (AMY) and leucine aminopeptidase (LAP) are polymorphic enzymes with single-banded homozygotes and double-banded heterozygotes. Variant band positions are designated by numbers which reflect their migration rate in the electrical field. Higher numbers indicate slower mobility. Electrophoretic results show AMY bands at six different positions, designated AMY^{1}, $AMY^{1.5}$, AMY^{2}, AMY^{4}, AMY^{6} and AMY^{8}. Similarly, the alleles controlling these variants are designated as Amy^{1}, $Amy^{1.5}$, etc. The three LAP variants are designated LAP^{2}, LAP^{4} and LAP^{6} and the controlling alleles are Lap^{2}, Lap^{4} and Lap^{6}.

The allelic frequencies of AMY (Table 1) and LAP (Table 2) were calculated directly from the number of individuals in each phenotypic (genotypic) class. Expected Hardy-Weinberg proportions were calculated and compared with those observed. The comparisons of observed and expected numbers were simplified by separately grouping all homozygotes and all heterozygotes. The observed numbers are extremely close to expectation (Table 1 and Table 2). Only two significant differences were found, both from homozygote excess.

When allelic frequencies are placed on a map of the collection localities, patterns of geographic variation are seen (Figures 1 and 2). One such pattern is a

TABLE 1

Collection data, Amy-*allele frequencies, numbers of individuals examined, and observed vs. expected numbers of homozygotes in collections of* P. badius

Collection data†	Amy-allele frequencies						No. examined	Homozygotes obs./exp.
	1	1.5	2	4	6	8		
4(69) Lake, Fla.	0	0	0	.348	.580	.071	56	28/26
4(70) Lake, Fla.	0	0	0	.403	.552	.045	67	35/33
5(69) Lake, Fla.	0	0	0	.184	.816	0	38	26/26
5(70) Lake, Fla.	0	0	0	.211	.789	0	76	50/50
5(71) Lake, Fla.	0	0	0	.110	.890	0	82	66/66
6(69) Orange, Fla.	0	0	0	.211	.789	0	57	39/38
6(70) Orange, Fla.	0	0	0	.327	.673	0	75	44/42
6(71) Orange, Fla.	0	0	0	.368	.632	0	95	55/51
11(70) Alachua, Fla.	0	0	.008	.415	.577	0	65	28/33
12(69) Hamilton, Fla.	0	.051	.123	.370	.456	0	69	31/24
13(70) Lanier, Ga.	0	.096	.123	.507	.274	0	73	26/26
14(69) Coffee, Ga.	0	.198	.028	.443	.330	0	53	25/18
15(69) Sampson, N.C.	.029	.144	.442	.144	.240	0	52	26/15**
15(70) Sampson, N.C.	0	.071	.321	.125	.482	0	28	11/10
16(69) Bladen, N.C.	0	.044	.272	.079	.622	0	57	27/26
17(70) Cumberland, N.C.	0	.061	.286	.133	.520	0	98	33/36
18(70) Collier, Fla.	0	0	0	0	1.00	0	5	5/5
19(70) Highlands, Fla.	0	0	0	.027	.973	0	73	69/69
21(70) Hernando, Fla.	0	0	.027	.312	.629	0	93	42/46
22(70) Marion, Fla.	0	0	0	.211	.789	0	43	39/38
23(70) Pender, N.C.	0	0	.285	.104	.611	0	72	33/34
24(70) Sampson, N.C.	0	0	.330	.266	.404	0	47	27/27
25(70) Aiken, S.C.	0	.092	.317	.225	.367	0	60	17/17
26(70) Moore, N.C.	0	.083	.250	.167	.500	0	30	9/11
28(70) Appling, Ga.	0	.038	.076	.152	.734	0	66	40/40
29(70) Bullock, Ga.	0	.013	.162	.253	.571	0	77	38/32
30(70) Calhoun, S.C.	0	0	.053	.278	.669	0	65	37/34
31(70) Darlington, S.C.	0	.078	.190	.164	.569	0	58	25/23
32(70) Richmond, N.C.	0	.014	.410	.055	.521	0	72	37/32
33(70) Moore, N.C.	0	.200	.182	0	.618	0	55	22/25
34(70) Aiken, S.C.	0	.058	.149	.260	.533	0	77	39/29
35(70) Jefferson, Ga.	0	.298	.175	.474	.053	0	114	28/39
36(70) Early, Ga.	0	.164	.404	.432	0	0	73	24/28
37(70) Decatur, Ga.	0	.571	.201	.228	0	0	92	33/39
38(70) Worth, Ga.	0	.500	.162	.323	.015	0	34	14/14
39(70) Worth, Ga.	0	.474	.289	.237	0	0	19	8/7
40(70) Worth, Ga.	0	.539	.078	.133	.250	0	64	28/24
41(70) Talbot, Ga.	0	.424	.102	.475	0	0	59	13/24
42(70) Sumter, Ga.	0	.377	.308	.315	0	0	65	19/21
43(70) Hancock, Ga.	0	.468	.101	.411	.019	0	79	38/21
44(70) Saluda, S.C.	0	.109	.219	.240	.432	0	96	42/30*
45(70) Pender, N.C.	0	0	.211	.422	.367	0	90	27/32

Collection data†	Amy-allele frequencies						No. examined	Homozygotes obs./exp.
	1	1.5	2	4	6	8		
46(70)Lee, N.C.	0	.224	.134	.091	.551	0	49	22/18
47(70)Allendale, S.C.	0	.019	.119	.487	.371	0	54	16/17
48(70)Colombia, Fla.	0	.065	.361	.157	.417	0	80	34/31
51(70)Dale, Ala.	0	.286	.197	.157	0	0	119	47/47
52(70)Covington, Ala.	0	.228	.430	.342	0	0	57	22/21
53(70)Russel, Ala.	0	.384	.080	.536	0	0	56	22/24
54(70)Barbour, Ala.	0	.265	.165	.570	0	0	185	73/78

† Collection data are organized according to site identification number, year of collection—19(), and county and state in which collection was made, respectively.
* 0.01 < P < 0.05.
** P < 0.01.

TABLE 2

Allelic frequencies, numbers of individuals examined, and observed vs. expected numbers of homozygotes in collections analyzed for Lap

Collection identification	Lap-allele frequencies			Number examined	Homozygotes obs./exp.
	2	4	6		
4(69)	0	1.000	0	101	101/101
5(69)	0	1.000	0	66	66/66
6(69)	0	1.000	0	80	80/80
11(70)	0	1.000	0	65	65/65
12(69)	0	1.000	0	108	108/108
13(70)	0	1.000	0	78	78/78
14(69)	0	1.000	0	96	96/96
15(69)	0	.931	.069	102	88/88
16(69)	0	.889	.111	90	70/71
17(70)	0	.992	.008	60	59/59
25(70)	0	.943	.057	52	46/46
29(70)	.031	.969	0	80	77/75
30(70)	0	.971	.029	52	49/49
32(70)	0	.911	.089	28	24/23
33(70)	0	1.000	0	55	55/55
34(70)	0	1.000	0	80	80/80
35(70)	.017	.983	0	120	116/116
36(70)	0	1.000	0	75	75/75
37(70)	.037	.963	0	95	90/88
38(70)	0	1.000	0	35	35/35
39(70)	0	1.000	0	20	20/20
40(70)	0	1.000	0	65	65/65
41(70)	.031	.969	0	65	65/61
42(70)	0	1.000	0	70	70/70
43(70)	0	1.000	0	80	80/80
44(70)	.016	.983	0	90	89/87
46(70)	.041	.958	0	48	44/44
51(70)	.062	.937	0	120	105/105
52(70)	.107	.893	0	56	48/46
53(70)	.116	.883	0	60	53/48
54(70)	.265	.734	0	132	108/108

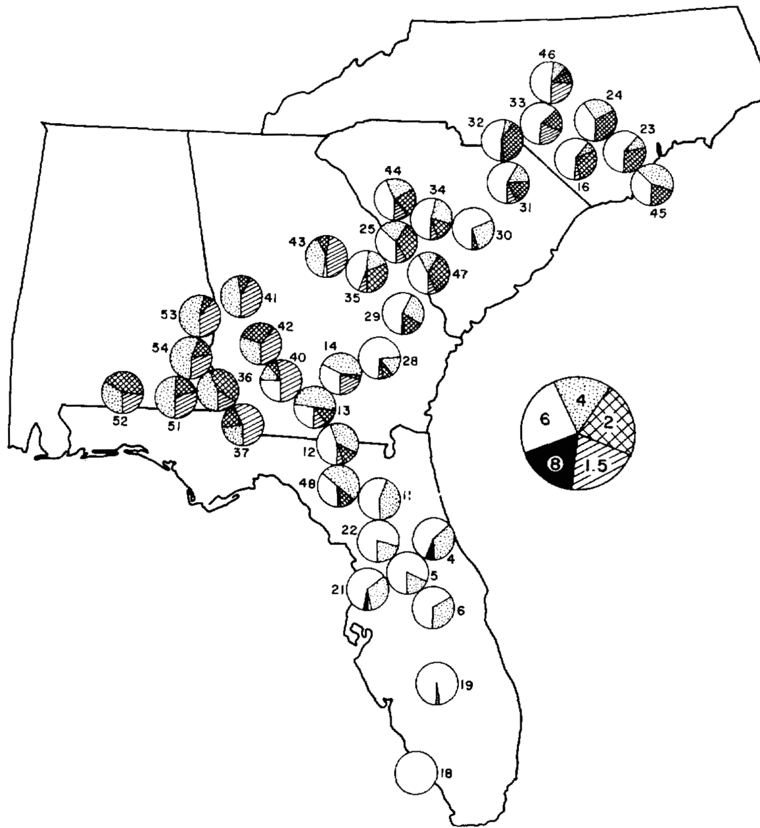

FIGURE 1.—*Amy*-allele frequencies as parts of circles at sites where collections were made. Each allele is represented by a different kind of shading. The number associated with each circle is the identification number for that collection site. To avoid crowding, a few sites have been omitted.

decrease in the frequency of Amy^6 from southern Florida and Georgia. Another is an east-west change in the frequency of Amy^6 from eastern Georgia into Alabama. In the LAP system, the less frequent variants, Lap^2 and Lap^6, are localized in different geographic regions.

The principal component analysis determined the nature and extent of the patterns of variability within the AMY and LAP systems. To avoid the possible effects of small gene samples, only those localities from which at least ten nests were sampled were included in the component analysis. This reduced the number of sites from 49 (listed in Table 1) to 43 (actually analyzed) for AMY and reduced the sites analyzed for LAP by two. The variance-covariance matrix for the *Amy* alleles was computed. Essentially all (99.8%) of the variability in the *Amy* gene frequencies is accounted for by the three components (1) corresponding to the three largest characteristic roots of the matrix. Although there is no *a priori* requirement for pattern in a multiple allelic system such as *Amy* (and

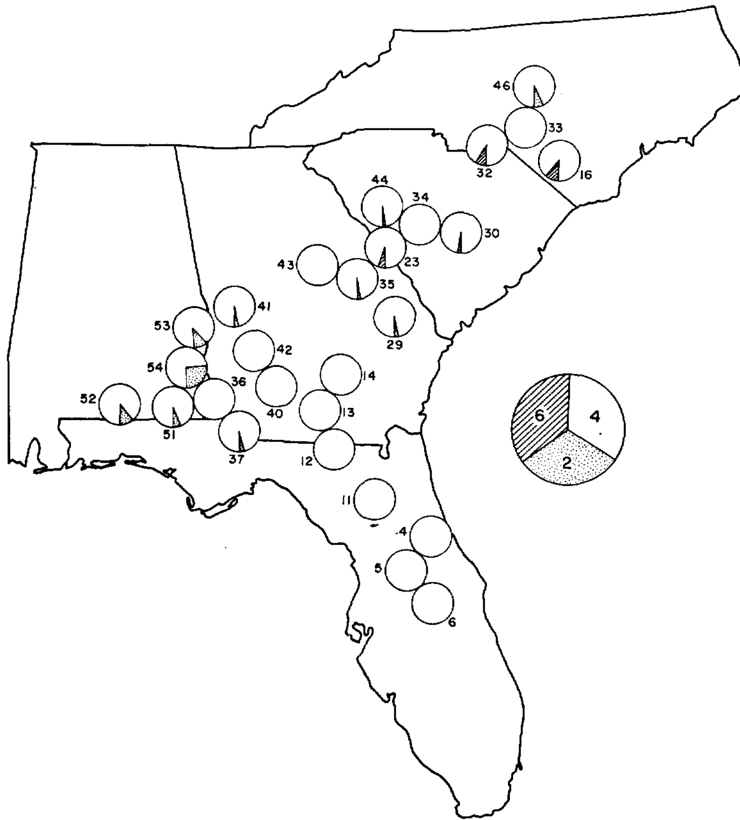

FIGURE 2.—*Lap*-allele frequencies at collection sites. Notations are similar to those of Figure 1.

pattern is not strongly evident from inspection of the pie diagrams in Figure 1) definite patterns are revealed in the components.

	Amy alleles							
	1	1.5	2	4	6	8	Percent of variation	
1st *Amy* component	−.0011	−.3946	−.1728	−.2867	+.8557	+.0062	73.5	
2nd *Amy* component	−.0088	−.0908	−.6928	+.7126	+.0568	+.0265	16.4	(1)
3rd *Amy* component	+.0046	−.7636	+.4966	+.3948	−.1196	+.0079	9.9	
							99.8	

As seen from the components above (1), most of the variation in *Amy* frequencies can be attributed to a large change in one direction of Amy^{6} accompanied by moderate change in the opposite direction of $Amy^{1.5}$, Amy^{2} and Amy^{4}. Addi-

tionally, there is a pattern (the second *Amy* component) composed mainly of changes in opposing directions of Amy^2 and Amy^4, and a pattern (the third *Amy* component) in which a change in $Amy^{1.5}$ is counterbalanced by a change in Amy^2 and Amy^4.

Two components, derived from the variance-covariance matrix for the *Lap* locus, describe all of the variation in the *Lap* frequencies (2):

	Lap alleles				
	2	4	6	Percent of variation	
1st *Lap* component	−.6875	+.7254	−.0350	82.1	(2)
2nd *Lap* component	−.4381	−.3758	+.8165	17.9	
				100.0	

Most of the variation in *Lap* frequencies is due to opposing changes in Lap^2 and Lap^4.

The relationships between the genetic components and a variety of environmental variables (Table 3 and 4) were examined. The component values were plotted against soil type, forest type, and proportion of land forested (from *A*

TABLE 3

Environmental data used in graphical examinations for correlation between environment and genetic component values

Coll. site	Environment†								
	ST	AF	FT	pH	OM	Ca	P	K	NO_3
4	E10	25	B	5.5	0.5	007	010	005	001
5	E10	50	A	6.9	1.3	100+	062	017	004
6	E10	50	C	—	—	—	—	—	—
11	U6	25	A	4.6	1.6	012	084	037	031
12	U6	75	A	4.7	1.1	008	056	018	024
13	U1	75	A	5.4	0.3	007	030	003	001
14	U1	75	A	—	—	—	—	—	—
15	S1	50	B	—	—	—	—	—	—
16	S1	75	B	—	—	—	—	—	—
17	U6	50	B	4.9	0.2	005	006	006	002
18	E1	25	E	—	—	—	—	—	—
19	E1	25	A	4.8	0.6	010	006	003	001
21	E10	50	A	6.4	1.3	100+	066	021	017
22	E10	50	A	—	—	—	—	—	—
23	H2	75	A	—	—	—	—	—	—
24	S1	50	D	—	—	—	—	—	—
25	U1	50	A	4.9	0.2	004	004	005	003
26	U6	75	A	—	—	—	—	—	—
28	U1	75	A	—	—	—	—	—	—
29	U1	50	A	—	—	—	—	—	—
30	U6	50	D	—	—	—	—	—	—
31	U6	50	A	—	—	—	—	—	—
32	U6	50	A	—	—	—	—	—	—

Coll. site	Environment†								
	ST	AF	FT	pH	OM	Ca	P	K	NO_3
33	U6	75	A	—	—	—	—	—	—
34	U1	50	C	4.7	0.9	016	032	016	029
35	U6	50	B	5.0	1.0	038	032	036	051
36	U6	50	D	5.2	1.3	034	042	028	063
37	U6	50	B	5.2	1.5	053	056	039	080
38	U6	25	A	—	—	—	—	—	—
39	U6	25	A	—	—	—	—	—	—
40	U6	25	A	4.7	1.2	059	048	041	051
41	U5	75	B	5.0	1.1	019	028	025	046
42	U6	50	B	—	—	—	—	—	—
43	U5	75	B	4.6	1.6	019	034	043	051
44	U6	50	A	5.0	1.3	016	046	029	033
45	S1	75	B	7.2	0.5	100+	100+	015	039
46	U5	50	B	—	—	—	—	—	—
47	U6	50	A	4.3	1.5	020	036	024	051
48	U6	50	B	—	—	—	—	—	—
51	U6	50	B	—	—	—	—	—	—
52	U6	50	B	—	—	—	—	—	—
53	U5	50	B	—	—	—	—	—	—
54	U6	50	B	—	—	—	—	—	—

† Data in the first three environmental columns were obtained from *A Forest Atlas of the South*. Soil type (ST) classifications are defined and mapped therein. Area forested (AF) is indicated as follows: 75–100% (75), 50–74% (50), 25–49% (25). Forest types (FT) are designated according to the predominant forms present; longleaf-slash pine (A), loblolly-shortleaf pine (B), oak-hickory (C), oak-gum-cypress (D), and unreproductive forest (E). The remaining environmental columns contain experimentally determined data as obtained from soil samples analyzed by the Soil Testing Division of the North Carolina Department of Agriculture. Hydrogen ion concentration is given in pH units; organic matter (OM) in percent transmission after acid extraction and the remaining in soil index values based on the relative requirements of a generalized plant, projected on a 0–100 scale.

Forest Atlas of the South, 1969). Some clustering of similar component values according to soil type was observed. All three of *Amy* principal components showed a different relationship to soil type (Figures 3, 4 and 5), but no strong patterns were suggested. Additional correlation was sought between experimentally determined environmental factors (pH, organic matter and elemental content) and component values. Again, no outstanding relationships were apparent. The strongest suggestions of association were between *Amy* component one and pH (Figure 6), and between *Amy* component one and organic content (Figure 7).

Another analysis of the relationships between environment and genotype utilized meteorological data and elevations obtained from U.S. Weather Bureau Records (*Climatography of the U.S.*). These data were "studentized" and then analyzed by the method of principal components. Since not all collections were analyzed for both *Amy* and *Lap*, two separate analyses were made, one for each enzyme. The variance-covariance matrices for the *Amy* environmental variables and for the *Lap* environmental variables were calculated. The corresponding component values are presented in (3) and (4), respectively. The order of the columns is elevation (EL), normal annual rainfall (ARAIN), mean January

TABLE 4

Environmental data on which principal component analysis was performed

Site no. and nearest weather station	Environment†					
	EL	ARAIN	MJAT	MJUT	MDMJAT	MDMJUT
4 Eustis	85	48.11	60.1	82.5	72.1	93.0
5 Eustis	85	48.11	60.1	82.5	72.1	93.0
6 Orlando AP	106	51.37	61.1	82.0	70.6	91.7
11 High Springs	75	53.51	56.7	81.5	68.7	93.2
12 Jasper 3SE	140	51.98	52.7	80.0	66.9	91.6
13 Alpaha	290	46.05	51.3	81.2	63.3	92.7
14 Douglas 2NNE	240	56.57	52.3	81.1	73.3	92.3
15 Willard 1N	51	48.97	47.1	79.4	57.7	90.0
16 Lumberton 6NW	132	42.48	45.2	79.8	56.6	90.4
17 Fayetteville 2SE	96	46.44	45.3	80.2	56.5	90.0
18 Naples	4	54.76	65.8	82.6	74.3	90.0
19 Lake Placid 25W	90	52.15	63.1	81.8	74.7	92.7
21 Brooksville Chi Hill	200	58.05	60.1	80.9	70.1	90.5
22 Ocala	86	53.95	58.5	81.4	71.1	92.3
23 Wilmington AP	30	51.29	48.2	80.3	56.7	89.4
24 Clinton	150	47.78	44.9	79.1	55.3	90.7
25 Aiken	527	43.56	47.2	80.3	58.8	92.6
26 Southern Pines 2W	497	50.67	44.3	78.9	54.3	91.2
28 Glenville	175	46.73	52.2	81.4	62.7	91.7
29 Brooklet 1W	190	43.82	50.2	81.6	62.7	91.4
30 Columbia AP	217	46.82	47.4	81.7	60.1	89.0
31 Bishopville	249	44.72	45.7	80.0	57.4	92.1
32 Hamlet	350	48.43	43.9	78.9	55.9	91.7
33 Pinehurst	548	49.88	43.9	79.2	53.3	91.5
34 Aiken	527	43.56	47.2	80.3	58.8	92.6
35 Louisville	335	43.33	48.3	81.0	60.5	94.1
36 Blakely	300	54.21	51.3	81.2	62.4	91.9
37 Bainbridge	120	51.59	52.4	81.7	66.2	92.5
38 Albany	210	47.84	51.1	82.3	63.3	93.5
39 Albany	210	47.84	51.1	82.3	63.3	93.5
40 Albany	210	47.84	51.1	82.3	63.3	93.5
41 Talbotton	700	51.30	47.7	79.9	59.6	91.5
42 Americus 4ENE	476	48.82	49.1	81.2	60.9	91.9
43 Milledgeville	320	44.90	46.4	80.9	57.8	91.8
44 Saluda	536	45.94	45.7	80.5	58.6	94.6
45 Maysville 6SW	44	56.65	45.7	77.3	57.1	89.0
46 Sanford 4ESE	369	50.53	42.3	78.7	54.4	90.2
47 Hampton	86	45.26	50.4	81.2	62.8	92.1
48 Lake City 2E	205	51.01	55.9	81.1	67.7	92.0
51 Ozark 6NNW	460	54.30	51.3	80.8	63.5	93.2
52 Andalusia 1NW	260	57.87	51.0	81.2	61.8	93.1
53 Tuskgee	440	47.90	49.3	80.9	56.8	92.2
54 Eufaula	220	52.50	48.8	80.8	61.9	93.1

† Data source was Climatography of the U. S. Weather reporting stations are those nearest the actual collection sites, and their position is indicated by the post office of their location. If they are not in cities, their rural locations are noted by distance (miles) and direction from the nearest post office; e.g., the weather station representing site number 12, Jasper 3SE, is three miles southeast of the Jasper post office. "AP" following a station name indicates that the weather station is located at the airport. Environmenal abbreviations are explained in the text. Units are feet for elevation, inches for rainfall and degrees Fahrenheit for temperature.

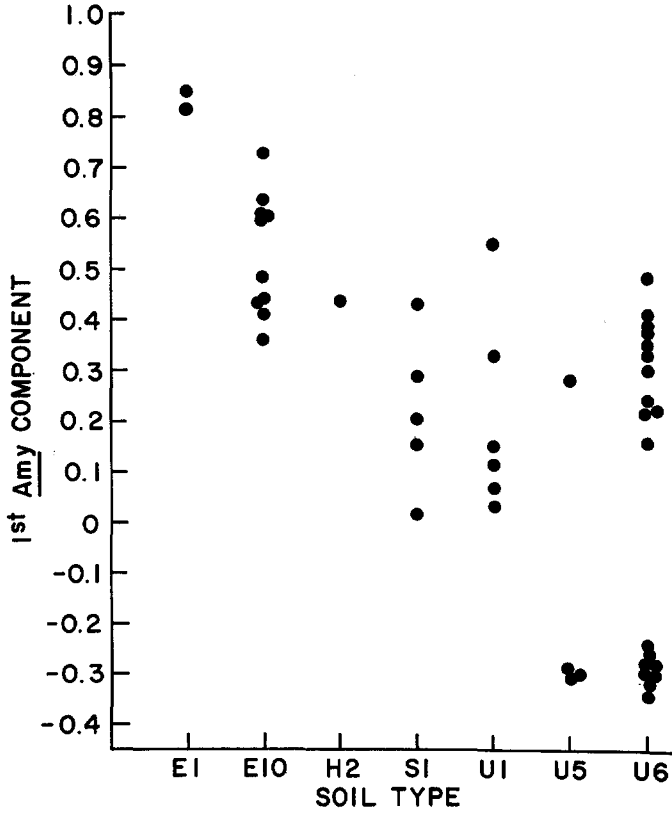

FIGURE 3.—First principal component values of the variation in *Amy*—allele frequencies in relation to soil type as defined and listed in *A Forest Atlas of the South*. Order does not reflect any obvious systematic variation in soil characters.

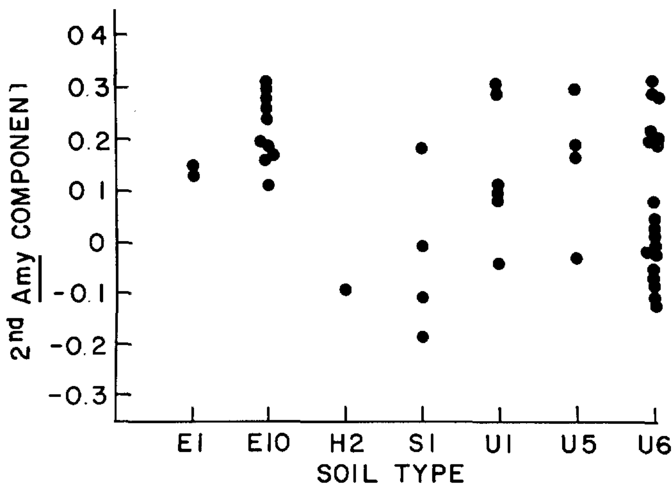

FIGURE 4.—Second principal component values of the variation in *Amy*-allele frequencies in relation to soil type.

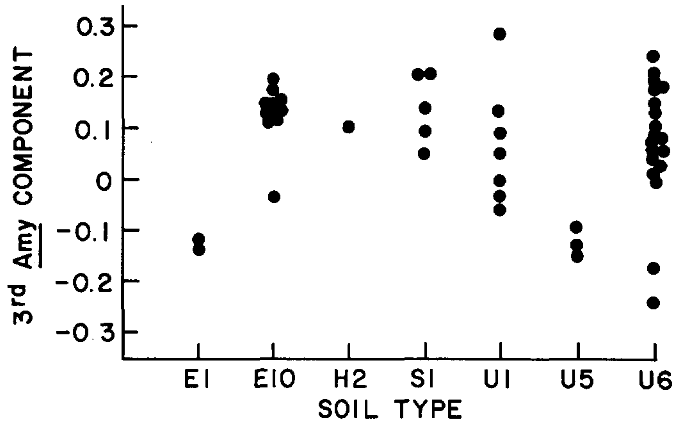

FIGURE 5.—Third principal component values of the variation in *Amy*-allele frequencies in relation to soil type.

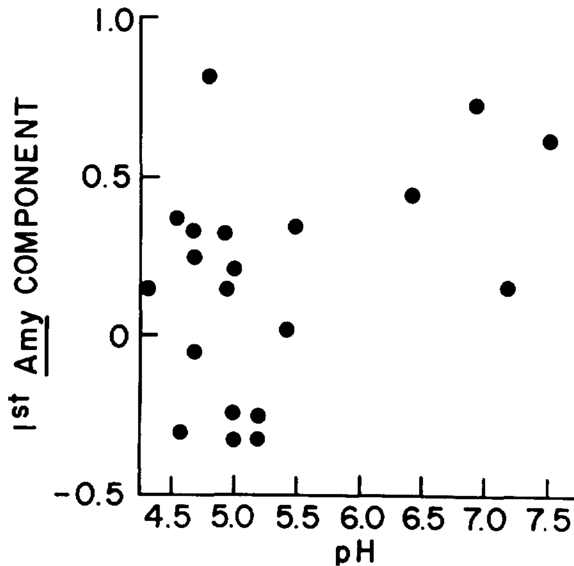

FIGURE 6.—First principal component values of the variation in *Amy*-allele frequencies in relation to soil pH.

temperature (MJAT), mean July temperature (MJUT), mean daily maximum January temperature (MDMJAT), and mean daily maximum July temperature (MDMJUT). The two sets are, as expected, quite similar. Three components account for more than 80% of the recorded climatographic data.

	Environmental factors (*Amy*)						
	EL	ARAIN	MJAT	MJUT	MDMJAT	MDMJUT	Percent of variation
1st envir. component	−.3106	+.1730	+.4819	+.4940	+.5749	+.2582	46.9

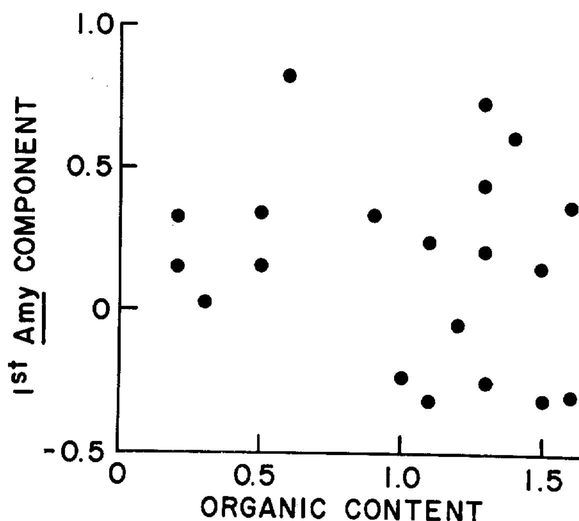

FIGURE 7.—First principal component values of the variation in *Amy*-allele frequencies in relation to the organic content of the soil.

2nd envir. component	+.4659	−.4841	−.1675	+.3059	−.0184	+.6532	24.3	(3)
3rd envir. component	+.5548	+.7553	+.0579	−.1969	+.0704	+.2732	15.2	
							86.4	

	Environmental factors (*Lap*)							
	EL	ARAIN	MJAT	MJUT	MDMJAT	MDMJUT	Percent of variation	
1st envir. component	−.3552	+.2662	+.4039	+.4910	+.5836	+.2411	44.4	
2nd envir. component	+.5741	−.0118	−.1872	+.1658	+.0229	+.7792	20.4	(4)
3rd envir. component	+.2917	+.8212	+.2512	−.4178	+.0026	−.0532	16.6	
							81.4	

The main pattern of variability described by the first component distinguishes low, wet, warm areas from those that are high, dry and cool—e.g., as coastal regions differ from the interior. The second and third components are less clear in geographic interpretations. For example, in the second component, high, dry regions with cool winters and warm summers are contrasted with low, wet regions with warm winters and cool summers.

The results of correlation analysis between the environmental components and the genetic components are given in (5). Out of fifteen total correlations, six are statistically significant, and four of the five genetic components are significantly correlated with at least one environmental component.

The maximum possible correlation between any genetic pattern and any environmental pattern in the data can be calculated by the method of canonical correlation analysis. This yields values of .8413** and .6941* for the *Amy*-environment and *Lap*-environment correlations, respectively. Thus, correlations greater than those in (5) can be obtained by choosing patterns other than those which entered into (5). The patterns which entered into (5) yield lower correlations since, unlike canonical correlation analysis, they were chosen to characterize the genetical and environmental data rather than to maximize the correlations.

	1st envir. compoent	2nd envir. component	3rd envir. component
1st *Amy* component	.3388*	−.2456	.4326**
2nd *Amy* component	.5640**	.0529	.2044
3rd *Amy* component	−.0396	−.3241*	.0033
1st *Lap* component	−.0768	−.0660	−.2348
2nd *Lap* component	.3982*	−.4074*	−.1775

(5)

* .01 < P < .05.
** P < .01.

Correlations also exist between the patterns in *Amy* and *Lap*. The results of this analysis are in (6):

	1st *Lap* component	2nd *Lap* component
1st *Amy* component	.3929*	.3019
2nd *Amy* component	.0860	−.5748**
3rd *Amy* component	−.1579	.2567

(6)

Comparisons of heat stability among the amylases were made to determine if variation in a functional property could be found in association with the structural variation that accounts for the electrophoretic differences. After treatment at 50°, all AMY bands had activity comparable to control gels. At 60° AMY^6 activity was markedly reduced, but all other isozymes displayed normal activity. The 70° treatment resulted in the reduction of activity of AMY^2 and AMY^4 while $AMY^{1.5}$ remained essentially normal. All AMY bands were reduced in activity after the 80° treatment. Thus, enzymatic activity was affected by heat in the order $AMY^6 > AMY^2 \approx AMY^4 > AMY^{1.5}$.

Thermal stability of enzymes could be important to the survival of the ants in their natural environment. Soil surface temperatures in excess of 140° F (60° C) have been reported at nest sites of *P. barbatus* in southern Texas (Box 1960). At the same latitude in Florida, similar temperatures would be expected. The geographic distribution of AMY phenotypes, however, is inconsistent with a hypothesis that heat is an important selective factor for the *Amy* locus. The most heat-labile form, AMY^6, is of highest frequency in the south. Therefore, if selection is important in maintaining the *Amy* gene frequencies, it would appear likely that the differences in heat stability among the forms are either unim-

portant or compensated by associated differences more important than stability to heat. The demonstration of a functional difference is central to the neutrality-selection question. The hypothesis of selective maintenance requires the existence of functional differences; the neutrality hypothesis tolerates functional differences but only if they are biologically ineffective. For most cases of naturally occurring variation, it remains to be shown the degree to which experimentally determined functional differences are meaningful to the life of the organisms.

If environmental factors directly or indirectly influence the relative fitness values of different isozyme genotypes, correlations like those observed are expected. If the variation is neutral, genetic drift and migration could result in correlated genetic and genetic-environmental patterns. In the absence of definite data, a few examples of genetic-genetic and genetic-environmental correlation do not provide support for either hypothesis. The number of examples is, however, increasing. In addition to the correlations presented here, others include the association between certain chemical constituents of cactus and isozyme gene frequencies in *Drosophila pachea* (Rockwood-Sluss, Johnston and Heed 1973), correlations of the variation in lactic dehydrogenase with geographic variation in *Pimephales promelas* (Merritt 1972), and correlations between genetic isozyme patterns and climatographic patterns in *P. barbatus* and *D. melanogaster* (Johnson *et al.* 1969; Johnson and Schaffer 1973). If the hypothesis of neutrality is correct, it is puzzling that the chance events required by the hypothesis continue to follow, consistently, geographic and environmental patterns of variability.

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