ISOZYME VARIATION IN NATURAL POPULATIONS OF *DROSOPHILA BUZZATII*

J. S. F. Barker and J. C. Mulley*
Department of Animal Husbandry, The University of Sydney, N.S.W. 2006 Australia

Received July 7, 1975. Revised October 24, 1975

Since the first demonstration that natural populations of *Drosophila* are polymorphic for a high proportion of protein and enzyme loci (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966; Johnson et al., 1966), similar high levels of electrophoretically detectable genetic variation have been found for many species (e.g., Johnson, 1974; Lewontin, 1974; Selander and Johnson, 1973).

The mechanisms by which this variation is maintained within populations and transformed into variation between populations remain uncertain. Recent experimental studies of natural populations have concentrated on the question as to whether the variation is maintained through some form of selection or whether it is selectively neutral. Some form of selection would be indicated, although not necessarily for the marker isozyme locus, if the frequency of a particular allele were found to vary consistently with change in some environmental component. Thus a number of attempts have been made to detect such associations (e.g., Johnson and Schaffer, 1973; Kojima et al., 1972; Rockwood-Sluss et al., 1973; Tomaszewski et al., 1973) or associations between heterozygosity and environmental variability (Bryant, 1974*a*,*b*).

However, most *Drosophila* species show a relatively uniform spatial pattern of gene frequencies, even across wide climatic distributions. This uniformity could be due to the unifying force of migration opposing differential selection in different populations, or to similar selective forces throughout the distribution, i.e. a function of the physiological properties of the molecules, or of the ecology of the species (breeding and feeding sites, nutritional requirements, behavioural responses to environmental variation), or to selective neutrality and migration. We would emphasize that "the relative importance of selective forces in maintaining even stable allele frequencies is difficult to determine until information is available on the ecology of the species and the actual function of the allozymes in relation to the physiology of the individual organism" (Rockwood-Sluss et al., 1973).

Therefore our aim was not merely to categorise the genetic variation in yet another species of *Drosophila*. *Drosophila buzzatii* was chosen because of its known occurrence over a wide distribution in spatially distinct populations, its known breeding and feeding site, and, consequently, its potential for the study of selective forces acting in natural populations. This paper considers the observed low level of genic variation, and theoretical aspects of the maintenance of this variation. Later papers will consider genotype-environment associations, further studies of spatial and temporal variation in *D. buzzatii*, and similar studies of the related cactophilic species *D. aldrichi*, which we have found in association with *D. buzzatii* in many localities (Mulley and Barker, in preparation).

Ecology and History of *D. buzzatii* in Australia

D. buzzatii, a member of the *mulleri* subgroup of the *repleta* group, is presumed to have originated in Argentina in association with one or more species of the cactus genus *Opuntia*, and to have spread to various parts of the world with these *Opuntia* species (Carson and Wasserman, 1965).

* Present address: School of Biological Sciences, University of Sydney.

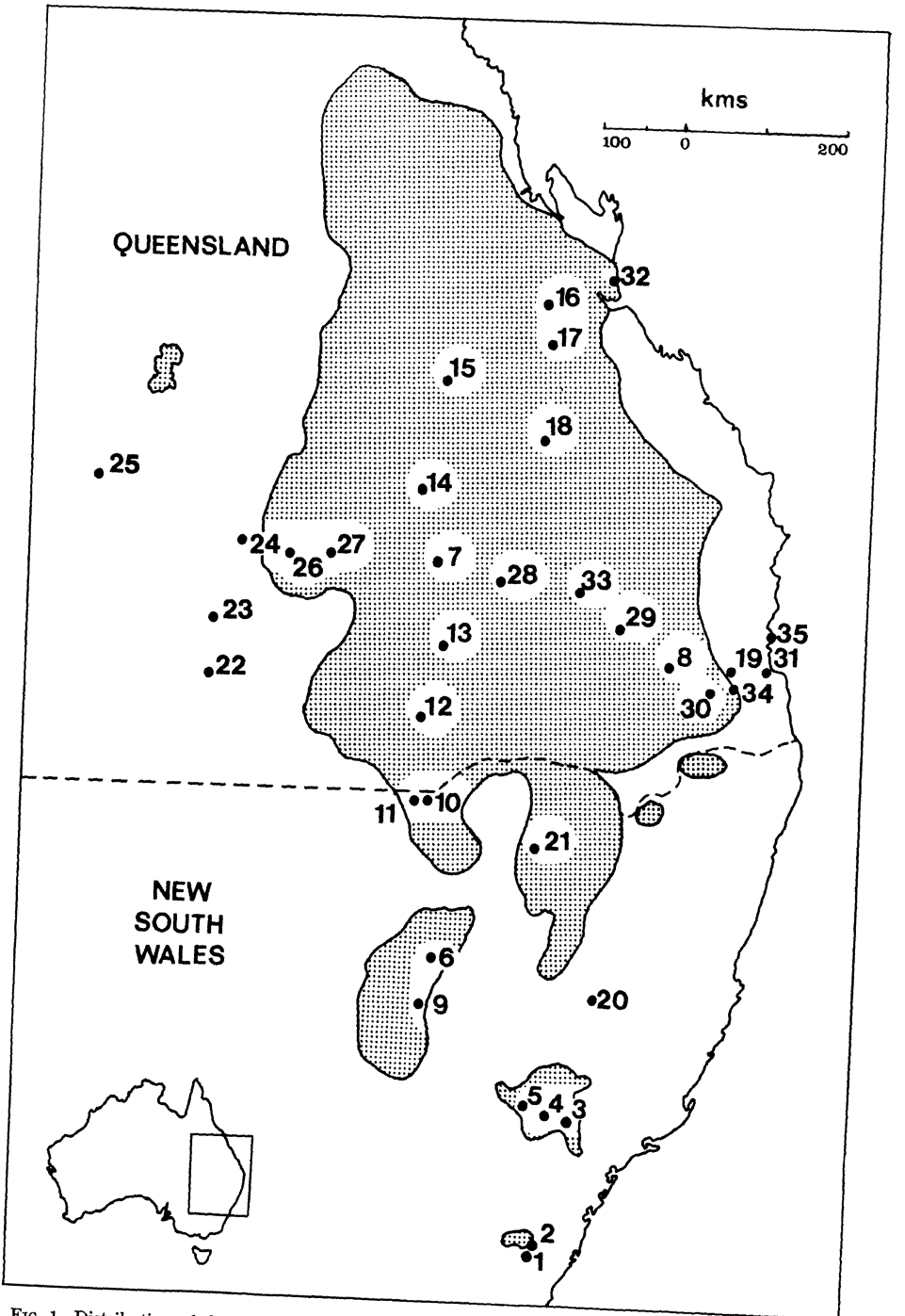

FIG. 1. Distribution of the main *Opuntia* infestations in 1920 (shaded areas), and the localities from which *D. buzzatii* populations were sampled.

It is apparently specific to the cactus niche. Our field observations show that larvae, pupae and adults inhabit rotting *Opuntia* cladodes and fruits and these are the only known breeding sites under natural conditions. Rot pockets in the cladodes develop from bacterial infections that follow physical damage to the plant normally caused by the burrowing activities of larval *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae). Our extensive collecting has failed to detect *buzzatii* from anywhere but the immediate vicinity of *Opuntia*. Furthermore, the worldwide distribution of *buzzatii* corresponds with the worldwide distribution of *Opuntia* (Carson and Wasserman, 1965; Mann, 1970; Wasserman, 1954). This evidence supports the view of absolute association under natural conditions. The distribution of *buzzatii* therefore is primarily determined by the *Opuntia* distribution, although it may be restricted within this by climatic or other environmental variables.

Nine species of cactus became major pests in Australia, and their colonization and final distribution have been documented by Mann (1970). One of these (*Opuntia monacantha*) arrived in Australia with the first European settlers in 1788—brought from Argentina together with cochineal insects in the hope of establishing a cochineal industry. No records exist as to when the other species were introduced. The two major prickly pear species (*O. inermis* = *O. bentonii* in the U.S., and *O. stricta*) are native to the Gulf of Mexico region (Texas, Louisiana, Florida). *O. inermis* is common around Galveston and *O. stricta* occurs in Florida and around Valparaiso, Chile. These were ports of call for 19th century sailing ships and it is reasonable to assume that the *Opuntia* were introduced as botanical curiosities from these and other American ports.

The prickly pear infestation in Australia was out of control by 1870, due to the absence of natural enemies. When the biological control program started in 1920, about 60,000,000 acres were affected and about half of this area was covered by pear so dense that the land was useless (Fig. 1). *C. cactorum* was introduced in 1925, and by 1940, complete control of the pear had been achieved, with both host and parasite continuing to exist at equilibrium. In addition to the areas shown in Figure 1, isolated patches of *Opuntia* occur in southern N.S.W. and Victoria. Thus the present Opuntia distribution is within the limits of the original infestation, but the species occur as an island distribution, with island size ranging from less than one hectare to a few hundred hectares.

The *buzzatii* distribution is assumed to be the same as the *Opuntia* distribution—i.e. an island distribution with considerable variation in island size and *buzzatii* population size, variation in inter-island distance, a number of peripheral isolate populations, some variation among islands in ecology (both in *Opuntia* species and in other flora), with the whole distribution extending over a wide geographical area and climatic range. Given isolation between the *buzzatii* populations inhabiting these islands (particularly between small peripheral isolates), drift and/or selection could lead to genetic differentiation among populations. However, the degree of genetic isolation, as distinct from observed spatial isolation, remains unknown.

The other important unknown is the time of introduction of *D. buzzatii* to Australia, and the size of its founding population. It could have accompanied any one or more of the *Opuntia* introductions to Australia. As these were presumably healthy plants with no rot, any such introduction probably would have been in fruit. Further, as bacterial rots were apparently unknown before the biological control program, any *buzzatii* population could be maintained only in fruits. As *Opuntia* fruit only once per year, this would seem unlikely. Nevertheless, Carson and Wasserman (1965) raised *D. buzzatii* from decaying fruit of *O. ficus-indica* in Victoria, but did not raise any from decaying cladodes. However, we have recently collected from *O. ficus-indica*

stands in Victoria and South Australia, and have raised *buzzatii* from both decaying cladodes and fruit. In addition, as we have never raised *buzzatii* from fruit of other *Opuntia* species, maintenance of *buzzatii* populations is apparently dependent on cladode rot.

Alternatively, *buzzatii* could easily have been introduced in some of the 1230 crates of *Opuntia* imported during the biological control program (Mann, 1970). Much of this was decaying material carrying the immature stages of many insect species. This mode of introduction seems more likely. In 1924–25, as part of the biological control programme, all species of flies found breeding in decaying pear at a number of localities in Queensland were identified. *D. melanogaster* was found at all localities throughout the year, but was the only Drosophilid recorded (Haseler, pers. comm.).

Materials and Methods

Sample preparation.—Each fly was squashed in supernatant buffer by a power grinder and centrifuged in microsample tubes. Either 15 or 25 μl of supernatant solution was used depending on whether the sample was to be split. Supernatant buffer consisted of 0.1 M tris-borate pH 8.9 and 1 per cent bromophenol blue indicator in the ratio of 6 : 1. This solution was then added to 50 per cent aqueous sucrose in the ratio of 7 : 2.

Polyacrylamide gel electrophoresis.—Electrophoresis was performed in multicell Gradipore units using continuous gradient polyacrylamide gels. Gels were run at 300 V for two hours in 0.1 M tris-borate buffer at pH 8.9. The buffer was cooled by circulation through heat exchange bags in ice. Gel boxes contained buffer with 1 gm/litre of EDTA; or, if the assays required cations, EDTA was substituted by 0.5 gm/litre of $MgCl_2$.

Starch gel electrophoresis.—Starch gel electrophoresis was done using Connaught starch (29 g/250 ml). Each homogenate was applied to 6×4 mm filter paper (Beckman No. 319329) which was inserted about 3 cm from the cathodal end of the gel. Electrophoresis was performed at 200 V for 4 hours. During electrophoresis the gels were encased in frozen cooler-bricks to prevent overheating.

The following buffer systems were used for starch gel electrophoresis. *Buffer I*: Discontinuous tris-citrate (Poulik, 1957). *Buffer II*: Continuous tris-citrate, pH 7.0 (Ayala et al., 1972). *Buffer III*: Continuous tris-EDTA-borate, pH 8.0 (Shaw and Koehn, 1968). NADP (7.5 mg) was added to the starch for the detection of *G-6-Pdh* and *6PGdh*. The enzymes studied, locus analysed, lifestage studied and routine gel media are indicated in Table 1.

Staining methods.—Adenylate kinase: 90 mg glucose, 20 mg $MgCl_2$, 7.5 mg NADP, 20 mg ADP, 25 mg NBT, 40 units glucose-6-phosphate dehydrogenase, 160 units hexokinase, 50 ml tris-HCl buffer pH 7.1 (0.05 M). After incubating for 1 hour, add 3 mg PMS. Amylase: Stand gels in 1% hydrolysed starch at 5°C for 2–3 hours for *Amy-2* or overnight for *Amy-1*, transfer to tris-HCl buffer (0.2 M, pH 7.4), add 0.5 ml $CaCl_2$ (1 M) and incubate at 37°C for 30 minutes. Stain in iodine (0.1 N) for 2 minutes. Dehydrogenases (general): Tris-HCl (0.2 M, pH 8.5), substrate, coenzyme (7.5 mg NAD or 3.75 mg NADP), 12.5 mg NBT, 1 mg PMS and incubate at 37°C in darkness until bands appear. Substrate concentrations for NAD linked dehydrogenases: Alcohol dehydrogenase: 1 ml isopropanol. Aldehyde oxidase: 1 ml acetaldehyde and 1 ml benzaldehyde. α-Glycerophosphate dehydrogenase: 20 mg DL-α-glycerophosphate. Lactate dehydrogenase: 0.5 ml DL-lactic acid. Malate dehydrogenase: 25 mg sodium hydrogen malate. Octanol dehydrogenase: 1 ml octanol. Xanthine dehydrogenase: 50 mg hypoxanthine. Substrate concentrations for NADP linked dehydrogenases: Glucose-6-phosphate dehydrogenase: 50 mg glucose-6-phosphate. Isocitrate dehydrogenase: 150 mg DL-isocitrate, 20 mg $MnCl_2$. Malic

TABLE 1. *The enzymes studied, the corresponding locus, life stage analysed and routine gel media.*

	Enzyme	Locus	Stage analysed	Gel*
1	Adenylate kinase-1	*Adk-1*	adults	II
2	Adenylate kinase-2	*Adk-2*	adults	II
3	Alcohol dehydrogenase-1	*Adh-1*	larvae	I
4	Alcohol dehydrogenase-2	*Adh-2*	larvae	I
5	Aldehyde oxidase	*Ao*	adults	A
6	Amylase-1	*Amy-1*	larvae	A
7	Amylase-2	*Amy-2*	larvae	A
8	Esterase-1	*Est-1*	adults	A
9	Esterase-2	*Est-2*	adults	II
10	Esterase-3	*Est-3*	adults	A
11	Glucose-6-phosphate dehydrogenase	*G-6-pdh*	adults	III
12	Glucose phosphate isomerase	*Gpi*	adults	A
13	α-Glycerophosphate dehydrogenase	*α-Gpdh*	adults	III
14	Hexokinase-1	*Hk-1*	adults	II
15	Hexokinase-2	*Hk-2*	adults	II
16	Hexokinase-3	*Hk-3*	adults	II
17	Isocitrate dehydrogenase	*Idh*	adults	II
18	Lactate dehydrogenase	*Ldh*	pupae	A
19	Larval protein	*Lp*	larvae	A
20	Leucine-amino peptidase-1	*Lap-1*	larvae	A
21	Leucine-amino peptidase-2	*Lap-2*	larvae	A
22	Malate dehydrogenase	*Mdh*	adults	A
23	Malic enzyme	*Me*	adults	A
24	Octanol dehydrogenase	*Odh*	larvae	I
25	Phosphoglucomutase	*Pgm*	adults	A
26	6-Phosphogluconate dehydrogenase	*6-Pgdh*	adults	III
27	Pyranosidase	*Pyr*	adults	II
28	Tetrazolium oxidase	*To*	adults	III
29	Xanthine dehydrogenase	*Xdh*	adults	A

* A = Routinely stained on polyacrylamide gels (remainder on starch); I = Starch buffer I; II = Starch buffer II; III = Starch buffer III.

enzyme: 25 mg sodium hydrogen malate. 6-Phosphogluconate dehydrogenase: 50 mg 6-phosphogluconic acid. *Esterase*: Lower pH of gel in phosphate buffer (0.1 M, pH 6.5) for 30 minutes, 10 mg α-naphylacetate (50 : 50 acetone : H_2O), 10 mg β-naphylacetate (50 : 50 acetone : H_2O), 1 ml propanol and 25 mg fast red after 30 minutes. Glucose phosphate isomerase: Tris-HCl (0.2 M, pH 8.5), 100 mg fructose-6-phosphate (sodium salt), 1 ml NADP, 5 units *G-6-Pdh*, 12.5 mg NBT, incubate at 37°C for 30 minutes in darkness, 1 mg PMS and continue incubation in dark until bands appear. Hexokinase: 90 mg glucose, 20 mg glucose, 20 mg $MgCl_2$, 25 mg ATP, 7.5 mg NADP, 25 mg NBT, 40 units *G-6-Pdh*, 50 ml tris-HCl buffer (0.05 M, pH 7.1), 3 mg PMS after 1½ hours. Larval proteins: Lower pH of gel in 12.5% trichloroacetic acid for 2 hours, transfer to fresh trichloroacetic acid, add 2.5 ml of 1 per cent coomassie blue and stand overnight at room temperature. Leucine-amino peptidase: Lower pH of gel in tris-maleate (0.2 M, pH 5.2) for 30 minutes, transfer to fresh buffer, 20 mg L-leucyl-β-napthylamide-HCl and 25 mg fast black K after 30 minutes. Phosphoglucomutase: See Mulley (1973a). Pyranosidase: See Mulley (1973b), but modified to use buffer system II. Tetrazolium oxidase: 12.5 mg NBT, 1 mg PMS and leave in light.

The banding of *Adk-1, Amy-2*, often *Est-3*, and sometimes *Lap-2*, *6-Pgdh* and *Xdh* was somewhat diffuse, but no evidence of variation was observed. *Idh* and *Me* were often difficult to stain. Nomenclature was

TABLE 2. *Allelic frequencies at the* Est-1 *locus.*

	Locality	Collection Date	Genes Sampled	*Est-1*^{a}	*Est-1*^{b}	*Est-1*^{c}	Heterozygosity Obs.	Heterozygosity Exp.
1	Cawdor	3.12.71–25.4.72	186	.20	.80	--	.34	.33
2	Cobbitty	12.2.72–25.4.72	356	.13	.87	--	.25	.22
3A	Warkworth	12.12.70	44	.16	.84	--	.14	.27
3B	Warkworth	20–21.12.71	334	.18	.81	.02	.29	.32
3C	Warkworth	10–11.4.72	268	.16	.80	.04	.23	.33***
3D	Warkworth	29.4.73	382	.17	.76	.07	.35	.39*
3E	Warkworth	29.11.73	214	.19	.78	.03	.23	.36***
4A	Woodlands Hill	3–4.2.72	270	.12	.88	.004	.19	.22
4B	Woodlands Hill	22.2.72	210	.17	.82	.01	.24	.29*
4C	Woodlands Hill	8–10.4.72	268	.11	.86	.03	.24	.25
5	"Yarrawonga"	7.4.72	290	.18	.80	.02	.32	.33
6A	Kenebri	29–30.1.73	294	.19	.79	.01	.27	.33*
6B	Kenebri	21.4.73	334	.26	.61	.13	.50	.54**
6C	Kenebri	14.8.73	280	.27	.65	.08	.41	.49*
6D	Kenebri	28.11.73	130	.18	.67	.15	.54	.50
6E	Kenebri	15.8.74	242	.20	.76	.04	.33	.37
7A	Roma	1.2.73	254	.24	.70	.06	.33	.45**
7B	Roma	18.8.73	308	.32	.62	.06	.42	.51*
7C	Roma	26.11.73	176	.16	.80	.03	.26	.33*
7D	Roma	17.8.74	208	.24	.71	.05	.37	.43*
8	Toowoomba	6–7.2.73	424	.18	.74	.09	.32	.42***
9	"Warrawong"	13.8.73	78	.24	.74	.01	.31	.39
10	"Cleveland" East	15.8.73	98	.16	.81	.03	.22	.32*
11	"Cleveland" West	15.8.73	96	.27	.59	.14	.35	.56**
12A	"Moonie Ponds"	16.8.73	158	.22	.73	.04	.30	.41**
12B	"Moonie Ponds"	16.8.74	124	.21	.77	.02	.37	.37
13	Donga Creek	17.8.73	306	.34	.61	.05	.44	.51*
14	Injune	18.8.73	152	.41	.51	.08	.38	.56***
15A	"Glen Elgin"	19.8.73	166	.40	.55	.05	.45	.54
15B	"Glen Elgin"	18.8.74	118	.23	.75	.03	.37	.39
16A	Westwood	19.8.73	48	.08	.81	.10	.29	.32
16B	Westwood	22.8.74	188	.35	.62	.03	.43	.50
17	"Dixalea"	20.8.73	48	.19	.69	.13	.46	.48
18A	Theodore	20.8.73	44	.25	.68	.07	.32	.47
18B	Theodore	23.8.74	250	.24	.74	.01	.25	.39***
19	Borallon	21.8.73	52	.37	.63	--	.35	.46
20	Tamworth	22.8.73	140	.23	.73	.04	.26	.42***
21	Terry Hie Hie	20.9.73	180	.13	.87	--	.19	.22
22	"Yarmouth"	23.11.73	108	.31	.68	.01	.39	.44
23	Murweh	24.11.73	14	.50	.50	--	.43	.50
24	Charleville	24.11.73	50	.26	.70	.04	.36	.44
25	"Bulloo Lakes"	25.11.73	56	.27	.73	--	.39	.39
26	Angellala	26.11.73	160	.17	.77	.06	.29	.38**
27	Mungallala	26.11.73	186	.27	.69	.04	.42	.45
28	Jackson	26.11.73	186	.24	.67	.09	.47	.48
29	Dalby	27.11.73	156	.19	.73	.08	.32	.42*
30	Grandchester	27.11.73	162	.28	.61	.10	.46	.53*
31A	Hemmant	27.11.73	216	.23	.75	.02	.36	.38
31B	Hemmant	12.5.74	318	.26	.69	.05	.40	.45
31C	Hemmant	25.8.74	102	.24	.72	.05	.41	.43
32	Emu Park	22.8.74	190	.11	.86	.03	.20	.24
33	Chinchilla	24.8.74	222	.14	.85	.02	.30	.26
34	Moreton	24.8.74	202	.25	.68	.06	.37	.47*
35	Deception Bay	5.8.74	144	.28	.69	.03	.28	.44**

* Significant departure from Hardy-Weinberg equilibrium, $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 3. *Genetic tests of crosses between homozygous strains showing F_1 genotype and F_2 observed and expected genotypic numbers. Variant alleles are designated Fast or Slow in mobility relative to the standard* (Est-1^b, Est-2^d, Adh-1^b, Pyr^a, Ao^a, Pgm^b).

Variant*	Mobility Relative to Standard	F_1** Slow/Fast	F_2 Fast	F_2 Slow/Fast	F_2 Slow	Chi-Square
Est-1^a	Fast	10	21(20)***	38(40)	21(20)	0.2
Est-1^c	Slow	10	10(10)	22(20)	8(10)	0.6
Est-2^a	Fast	10	15(20)	40(40)	25(20)	2.5
Est-2^b	Fast	10	13(10)	22(20)	5(10)	3.6
Adh-1^c	Slow	10	4 (5)	8(10)	8 (5)	2.4
Pyr^b	Slow	10	11(10)	21(20)	8(10)	0.5
Ao^b	Slow	10	24(20)	44(40)	12(20)	4.4
Pgm^a	Fast	10	5 (5)	8(10)	7 (5)	1.2

* *Est-2*^{c}, *Adh-2*^{b} and *Amy-1*^{b} were not tested.
** 5 males, 5 females assayed for each cross.
*** Expected genotypic numbers are in parentheses.

standardised such that *Est-1* migrated faster than *Est-2*, and *Est-1*^{a} migrated faster than *Est-1*^{b}, etc.

The 35 localities sampled are shown in Figure 1, and their names, and dates of all collections, are given in Table 2. Collections made at different times at the same locality are designated by capital letters following the locality number. In most localities, only *O. inermis* was present. Locality 2 had only *O. monacantha*, locality 18 only *O. streptacantha*, localities 15 and 16 only *O. tomentosa* while localities 14, 17 and 30 had both *O. inermis* and *O. tomentosa*. These latter two species occurred together with *O. stricta* at locality 19, but no rots harbouring any life cycle stage of *buzzatii* were found in *O. stricta*. All collections prior to August, 1973 (except for localities 1 and 2) and the 3E collection were made from fermenting banana bait buckets placed near to *Opuntia* plants. Adults from localities 1 and 2 were caught in traps baited with heavily yeasted *Drosophila* medium. All later collections were made by aspiration of adults from rot pockets. In four collections (14, 15B, 16B, and 20) wild-caught adult numbers were supplemented by adult emergences from rot pockets brought back to the laboratory. Gene frequencies from wild-caught adults and such emergences were not significantly different and they have been pooled in the results.

As *D. buzzatii* can be reared in the laboratory on dead-yeast fortified medium, test matings were done for alleles that that accounted for the major portion of the variation (Table 3). None of the chi-square values are significant, and all loci conform with simple autosomal inheritance.

RESULTS

For the collections up to February, 1973, from localities 1–8, all 29 loci were surveyed. This preliminary investigation uncovered only six loci that were consistently variable—viz *Est-1*, *Est-2*, *Adh-1*, *Pyr*, *Ao* and *Pgm*. A single *Amy-1* variant was found in locality 4 (Table 4). In addition, *Adh-2* which was assayed together with *Adh-1*, was later found to be sporadically variable at low frequency throughout the total distribution (Table 5).

For the remaining "non-variable" loci, given the sample sizes studied (Table 6), the probability of undetected segregation was negligible. Alleles of very low frequency may have been missed when two to five samples were taken from each isofemale line that sometimes had been maintained in the laboratory for a few generations. Further, given the sample sizes studied for these 21 loci and for *Amy-1*, and the distribution of localities 1–8 in the total species distribution, these

TABLE 4. *Allelic frequencies at the* Amy-1 *locus.*

Locality	Genes Sampled	*Amy-1*^{a}	*Amy-1*^{b}
1	56 (6)†	1.0	..
2	80 (20)	1.0	..
3	120 (30)	1.0	..
4B	210 (53)	0.995	.005
4C	80 (40)	1.0	..
5	142 (15)	1.0	..
6	80 (20)	1.0	..
7	80 (20)	1.0	..
8	80 (20)	1.0	..
No. of wild genomes assayed*	448		

† No. of isofemale lines analysed given in parenthesis.

* = 2× (No. of isofemale lines).

TABLE 5. *Allelic frequencies at the* Adh-2 *locus.*

Locality	Genes Sampled	*Adh-2*^{a}	*Adh-2*^{b}	Heterozygosity Exp.
6B	180 (45)†	.994	.006	.012
7B	278 (39)	.996	.004	.007
7C	174 (44)	.983	.017	.034
14	70 (7)	.971	.029	.056
15A	160 (40)	.994	.006	.012
22	274 (27)	.985	.015	.029
29	150 (38)	.987	.013	.026

† No. of isofemale lines analysed given in parentheses.

22 loci were assumed invariant in localities 9–35. To the extent that any sporadically variable at low frequency, as found for *Adh-2*, average heterozygosity will be slightly underestimated. Including collections at different times from the same locality, a total of 50 populations were characterised for all seven variable loci. Additional gene frequency estimates were made for only some of these loci in the earlier collections, and a temporal study is continuing at locality 5.

The variable loci were usually analysed from wild-caught individuals, with the exception of *Adh-1* and *Adh-2* which were normally determined from larval progeny of wild-caught females because of the higher enzyme activity in larvae. Samples assayed from isofemale lines (for *Adh* and for other loci early in the study) are identified in Tables 7–11, where the number of isofemale lines assayed is given. Given the number of genes sampled and the number of isofemale lines in these tables, the average number assayed per isofemale line can be determined. Gene frequencies were estimated directly from the sample of individuals assayed. However, for statistical tests and counts of the numbers of wild genomes assayed, the latter was estimated (for loci other than *Adh*) as $2l$, where l is the number of isofemale lines. For *Adh*, where varying number of larvae per wild-caught female were assayed from different populations, the number of wild genomes was estimated as $4l$ where seven or more larvae were assayed per female, $3l$ for three to six larvae, and $2l$ for two larvae.

For the variable loci, and for each locality, the number of genes sampled, allelic frequencies, observed proportion of heterozygotes, and the proportion expected to be heterozygous on the assumption of Hardy-Weinberg equilibrium are given in Tables 2 and 7–11. Significant deviations from Hardy-Weinberg equilibrium are indicated—all but one (*Est-2*, locality 33) involved an observed deficiency of heterozygotes. In addition, for all loci there were generally more cases of heterozygote deficiency than of heterozygote excess. Further analysis was done for each locus by comparing the observed and expected numbers of heterozygotes, and counting the number of cases of observed heterozygote deficiency and excess. Cases where observed and expected numbers were equal or differed by less than unity were divided equally between the two groups. Sign tests then were done to determine if the numbers in the two groups differed significantly from equality. For all five loci, there were significantly more cases of heterozygote deficiency ($P < 0.01$ for *Est-1*, *Est-2*, *Pyr* and *Ao*, $P < 0.05$ for *Pgm*).

For the variable loci, numbers of populations and wild genomes sampled, average

TABLE 6. *Number of genes sampled for each of the 21 invariant loci. Where wild-caught individuals were not assayed, the numbers given are twice the number of isofemale lines assayed as a conservative estimate of the equivalent number of wild genomes.*

Locality	1	2	3	4	5	6	7	8	Total
Adk-1, 2	28	36	52	42	26	40	40	40	304
Amy-2	12	40	70	50	30	40	40	40	322
Est-3	106*	156*	118*	230*	252*	294*	254*	424*	1,834
G-6-pdh	28	40	100*	66*	30	40	40	40	384
α-Gpdh	32*	86*	152*	64*	50	40	40	40	504
Gpi	26*	44	38	88*	18	40	40	40	334
Hk-1, 2, 3	28	36	52	42	26	40	40	40	304
Idh	24	44	170*	120*	30	92*	40	216*	736
Lap-1	20	36	20	40	20	40	40	40	256
Lap-2	20	20	20	40	20	40	40	40	240
Ldh	28	34	18	40	26	40	40	40	266
Me	28	44	198*	124*	40	40	40	40	554
Mdh	66*	86*	158*	76*	50	40	40	40	556
Odh	28	26	82	24	28	40	40	40	308
6-Pgdh	28	34	52	40	28	40	40	40	302
To	48*	50*	58*	240*	50*	40	40	40	566
Xdh	56*	84*	98*	128*	126*	258*	88*	88*	926
Larval Protein	28	26	40	26	26	40	40	40	266
								Mean	427

* Assayed from wild individuals.

gene frequencies and variation statistics are summarized in Table 12. The heterozygosities given for each locus are the averages over populations of expected heterozygosities, and were not estimated from the average gene frequencies. The last line of the table gives the average heterozygosity, estimated as the average over all 29 loci. Average heterozygosity estimated for each population ranged from 0.051 (locality 2) to 0.078 (locality 6B), while the percentage of polymorphic loci ranged from 13.8 to 24.1 (1% criterion), and from 10.3 to 20.7 (5% criterion). Average estimates of the percentage of polymorphic loci (over the 50 populations assayed for all seven loci were 19.2 ± 0.35 and 15.3 ± 0.33 respectively.

Several generalisations arise from observations on the seven variable systems. Firstly, where a major allele exists, it remains the major allele in all localities. This was true for *Est-1*^{b}, *Pyr*^{a}, *Ao*^{a}, *Pgm*^{b} and *Adh-2*^{a}. Some spatial differentiation is obvious for *Est-1* (Table 2), but the other systems were nearer fixation, and show apparent interlocality stability. In addition, systems with no obvious major allele were present, viz. *Est-2* and *Adh-1*, and spatial genetic differentiation was observed in these (Tables 7 and 8). Differentiation was usually the result of minor gene frequency alteration, although some cases of large departures were noted. *Adh-1* in localities 31 and 35 (Table 8) and the temporal variation in *Est-1* at locality 7 (Table 2) are two such examples.

For each of the variable loci except *Adh-2*, the heterogeneity χ^2 testing differences among populations in gene frequency was highly significant ($P < 0.001$). Overall, there are no obvious major patterns of spatial variation, but some alleles show clinal variation. For example, for localities where collections were made in November to February, *Est-1*^{a} decreased significantly as longitude increased ($b = 0.0171 \pm 0.0069$, $P < 0.05$), while *Adh-1*^{b} in the

TABLE 7. *Allelic frequencies at the* Est-2 *locus.*

Locality	Genes Sampled	*Est-2*^{a}	*Est-2*^{b}	*Est-2*^{c}	*Est-2*^{d}	*Est-2*^{e}	Heterozygosity Obs.	Heterozygosity Exp.
1	178 (18)†	.11	.35	.44	.09	..	..	.66
2	368 (62)	.18	.48	.24	.10	..	..	.67
3B	214 (54)	.31	.24	.24	.21	..	..	.74
3C	240 (60)	.25	.31	.20	.24	..	..	.74
3D	318	.36	.24	.13	.26	..	.67	.72
3E	208	.32	.27	.13	.27	..	.62	.73**
4A	130 (13)	.23	.29	.26	.22	..	..	.75
4B	76 (19)	.26	.51	.11	.12	..	..	.64
4C	154 (39)	.31	.21	.23	.25	..	..	.75
5	178 (18)	.28	.34	.26	.11	..	..	.72
6A	198	.48	.26	.10	.15	..	.66	.66
6B	198	.46	.21	.11	.22	..	.64	.68
6C	234	.37	.22	.13	.27	..	.54	.72***
6D	128	.42	.31	.12	.15	..	.63	.69
6E	242	.38	.27	.10	.25	.004	.70	.71
7A	204	.33	.31	.15	.22	..	.67	.73**
7B	310	.30	.41	.09	.19	.01	.57	.70**
7C	176	.37	.35	.11	.17	.01	.70	.70
7D	208	.31	.29	.14	.26	..	.69	.73
8	208	.39	.34	.10	.17	..	.66	.69*
9	76	.30	.34	.12	.24	..	.66	.72
10	98	.44	.27	.14	.15	..	.65	.69
11	96	.22	.53	.16	.09	..	.50	.64*
12A	148	.39	.35	.16	.09	.01	.51	.69*
12B	124	.39	.32	.09	.20	..	.68	.70
13	274	.40	.31	.14	.16	..	.53	.70***
14	152	.36	.28	.13	.24	..	.68	.72
15A	162	.33	.32	.17	.18	.01	.73	.73
15B	118	.30	.25	.17	.29	..	.73	.74
16A	46	.48	.22	.15	.15	..	.65	.68
16B	188	.34	.40	.15	.11	..	.67	.69
17	48	.48	.31	.04	.17	..	.58	.64
18A	44	.32	.18	.16	.34	..	.59	.72
18B††	250	.41	.31	.09	.18	..	.67	.69
19	52	.44	.21	.17	.15	.02	.54	.71
20	138	.46	.24	.17	.12	..	.54	.68***
21	176	.30	.48	.03	.19	..	.73	.65
22	110	.33	.33	.17	.17	..	.56	.73
23	14	.43	.29	.21	.07	..	.43	.68
24	46	.35	.37	.02	.24	.02	.57	.68
25	56	.36	.38	.11	.16	..	.54	.69
26	158	.40	.35	.10	.15	..	.63	.68*
27	186	.39	.30	.11	.20	..	.63	.71
28	186	.40	.35	.09	.16	..	.65	.69
29	154	.42	.31	.08	.19	..	.56	.69
30	158	.34	.41	.13	.12	..	.62	.69
31A	220	.45	.20	.19	.17	..	.67	.70*
31B	302	.43	.24	.17	.16	.003	.64	.70
31C	102	.49	.22	.15	.15	..	.69	.67
32	190	.49	.26	.14	.11	..	.68	.66
33	222	.42	.29	.07	.22	..	.70	.69*
34	202	.39	.33	.10	.18	..	.68	.70
35	142	.25	.30	.20	.25	..	.72	.74

† No. of isofemale lines analysed given in parentheses.

†† An allele faster migrating than *Est-1*^{a} was found in this population at a frequency of 0.04.

* Significant departure from Hardy-Weinberg equilibruim, $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 8. *Allelic frequencies at the* Adh-1 *locus.*

Locality	Genes Sampled	*Adh-1*[a]	*Adh-1*[b]	*Adh-1*[c]	Heterozygosity Obs.	Heterozygosity Exp.
1	140 (14)†	--	.38	.62	--	.47
2	128 (13)	--	.35	.65	--	.46
3C	166 (42)	--	.42	.58	--	.49
3D	190 (48)	--	.49	.51	--	.50
3E	178 (45)	--	.57	.43	--	.49
4C	126 (13)	--	.34	.66	--	.45
5	144 (15)	--	.33	.67	--	.44
6A	260 (65)	--	.55	.45	--	.50
6B	180 (45)	--	.51	.49	--	.50
6C	162 (41)	--	.58	.42	--	.49
6D	160 (16)	--	.55	.45	--	.50
6E	128 (32)	--	.47	.53	--	.50
7A	288 (50)	--	.53	.47	--	.50
7B	278 (39)	--	.55	.45	--	.49
7C	174 (44)	.01	.47	.52	--	.51
7D	108 (18)	--	.54	.46	--	.50
8	192 (48)	--	.63	.38	--	.47
9	186 (19)	--	.55	.45	--	.49
10	146 (37)	.06	.53	.40	--	.55
11	132 (33)	.01	.61	.38	--	.48
12A	184 (46)	--	.57	.43	--	.49
12B	108 (18)	--	.44	.56	--	.49
13	148 (37)	--	.49	.51	--	.50
14	70 (7)	.03	.47	.50	--	.53
15A	160 (40)	--	.51	.49	--	.50
15B	88 (9)	.02	.55	.43	--	.52
16A	60 (6)	--	.32	.68	--	.43
16B	98 (25)	--	.57	.43	--	.49
17	100 (10)	--	.59	.41	--	.48
18A	88 (9)	--	.40	.60	--	.48
18B	136 (34)	--	.59	.41	--	.48
19	30 (3)	--	.57	.43	--	.49
20	72 (18)	--	.53	.47	--	.50
21	186	--	.49	.51	.37	.50**
22	274 (27)	--	.52	.48	--	.50
23	60 (6)	--	.52	.48	--	.50
24	30 (3)	--	.57	.43	--	.49
25	88 (9)	.01	.67	.32	--	.45
26	118 (30)	--	.46	.54	--	.50
27	186 (47)	--	.51	.49	--	.50
28	150 (38)	--	.65	.35	--	.46
29	150 (38)	--	.61	.39	--	.47
30	224 (56)	--	.57	.43	--	.49
31A	170 (43)	--	.86	.14	--	.23
31B	142 (36)	--	.84	.16	--	.27
31C	112	--	.74	.26	.34	.38
32	116 (29)	--	.66	.34	--	.45
33	132 (33)	--	.64	.36	--	.46
34	116 (29)	--	.51	.49	--	.50
35	84 (21)	--	.92	.08	--	.15

† No. of isofemale lines analysed given in parentheses.
** Significant departure from Hardy-Weinberg equilibrium, $P < 0.01$.

TABLE 9. *Allelic frequencies at the* Pyr *locus.*

Locality	Genes Sampled	Pyr^a	Pyr^b	Heterozygosity Obs.	Heterozygosity Exp.
1	150 (15)†	.92	.08	..	.15
2	144 (18)	.92	.08	..	.14
3C	148 (37)	.88	.12	..	.21
3D	324 (33)	.83	.17	..	.29
3E	214	.87	.13	.12	.22***
4C	156 (16)	.93	.07	..	.13
5	120 (15)	.88	.13	..	.22
6A	254	.94	.06	.03	.10**
6B	198	.85	.15	.22	.26
6C	236	.93	.07	.10	.13
6D	130	.91	.09	.18	.17
6E	242	.82	.18	.26	.30
7A	254	.94	.06	.09	.12
7B	306	.85	.15	.24	.26
7C	178	.92	.08	.10	.15
7D	208	.85	.15	.23	.26
8	424	.95	.05	.09	.10
9	76	.87	.13	.21	.23
10	94	.86	.14	.28	.24
11	92	.90	.10	.15	.18
12A	160	.89	.11	.18	.20
12B	124	.86	.14	.21	.24
13	306	.88	.12	.16	.21**
14	154	.94	.06	.10	.12
15A	164	.88	.12	.18	.20
15B	118	.86	.14	.17	.23*
16A	48	.96	.04	0	.08
16B	188	.84	.16	.26	.27
17	32	.94	.06	.13	.12
18A	42	.83	.17	.24	.28
18B	250	.84	.16	.23	.27
19	52	.87	.13	.19	.23
20	140	.97	.03	.03	.06
21	180	.92	.08	.16	.14
22	112	.90	.10	.13	.18
23	14	.93	.07	.14	.13
24	46	.91	.09	.17	.16
25	52	.83	.17	.19	.29
26	158	.92	.08	.10	.14
27	186	.96	.04	.04	.08
28	186	.85	.15	.23	.25
29	156	.90	.10	.17	.17
30	162	.93	.07	.09	.13
31A	186	.94	.06	.10	.11
31B	318	.95	.05	.09	.10
31C	102	.95	.05	.10	.09
32	190	.85	.15	.20	.26*
33	222	.85	.15	.26	.25
34	202	.86	.14	.26	.24
35	144	.84	.16	.21	.27

†No. of isofemale lines analysed given in parentheses.

* Significant departure from Hardy-Weinberg equilibrium, $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 10. *Allelic frequencies at the* Ao *locus.*

Locality	Genes Sampled	Ao^a	Ao^b	Heterozygosity Obs.	Heterozygosity Exp.
1	188	1.0	..	0	0
2	318	1.0	..	0	0
3B	178	.96	.04	.08	.08
3C	192	.97	.03	.06	.06
3D	192	.91	.09	.16	.16
3E	214	.98	.02	.05	.05
4A	186	.99	.01	.01	.01
4B	78	1.0	..	0	0
4C	270	.99	.01	.02	.02
5	136	.99	.01	.03	.03
6A	298	.98	.02	.03	.03
6B	188	.92	.08	.14	.15
6C	280	.96	.04	.08	.08
6D	130	1.0	..	0	0
6E	114	.94	.06	.09	.12
7A	254	.93	.07	.10	.14
7B	306	.93	.07	.08	.12
7C	178	.95	.05	.08	.10
7D	94	.98	.02	.04	.04
8	424	.94	.06	.10	.11
9	78	.94	.06	.03	.12
10	98	.84	.16	.12	.27*
11	96	.91	.09	.15	.17
12A	160	.91	.09	.13	.16
12B	48	.94	.06	.13	.12
13	312	.94	.06	.10	.11
14	154	.92	.08	.09	.15
15A	166	.92	.08	.11	.14
15B	20	1.0	..	0	0
16A	48	.96	.04	.08	.08
16B	142	.88	.12	.18	.21
17	32	.97	.03	.06	.06
18A	44	.91	.09	.09	.17
18B	126	.95	.05	.10	.09
19	52	.90	.10	.12	.17
20	140	.98	.02	.01	.04
21	180	.93	.07	.12	.13
22	110	1.0	..	0	0
23	14	.93	.07	.14	.13
24	50	1.0	..	0	0
25	56	.95	.05	.04	.10
26	140	.96	.04	.07	.07
27	162	.93	.07	.07	.14
28	158	.97	.03	.05	.05
29	156	.96	.04	.05	.07
30	162	.96	.04	.06	.08
31A	220	.99	.01	.03	.03
31B	318	.98	.02	.04	.04
31C	102	.99	.01	.02	.02
32	84	.99	.01	.02	.02
33	100	.93	.07	.10	.13
34	78	.96	.04	.08	.07
35	68	.96	.04	.09	.08

* Significant departure from Hardy-Weinberg equilibrium, $P < 0.05$.

TABLE 11. *Allelic frequencies at the* Pgm *locus.*

Locality	Genes Sampled	Pgm^a	Pgm^b	Pgm^c	Heterozygosity	
					Obs.	Exp.
1	88	--	1.0	--	0	0
2	252	--	1.0	--	0	0
3B	120 (30)†	.04	.96	--	--	.08
3C	94	.01	.99	--	.02	.02
3D	192	.02	.98	--	.04	.04
3E	212	.03	.97	--	.05	.06
4B	132 (33)	.01	.99	--	--	.02
4C	168	--	1.0	--	0	0
5	220	.02	.98	--	.05	.04
6A	298	.02	.98	--	.05	.05
6B	190	.05	.94	.01	.11	.12
6C	280	.03	.97	--	.04	.05
6D	130	.04	.96	--	.08	.07
6E	242	.03	.97	--	.05	.06
7A	254	.02	.98	--	.03	.03
7B	288	.01	.99	--	.02	.02
7C	176	.02	.98	.01	.05	.04
7D	208	.01	.99	--	.03	.03
8	424	.01	.99	--	.01	.02
9	76	.04	.96	--	.08	.08
10	82	.04	.96	--	.07	.07
11	94	.01	.99	--	.02	.02
12A	138	.01	.99	--	.03	.03
12B	124	.02	.98	--	.03	.03
13	266	.01	.99	--	.02	.01
14	78	.01	.99	--	.03	.03
15A	160	.01	.99	--	.01	.01
15B	118	.02	.98	--	.03	.03
16A	38	--	1.0	--	0	0
16B	188	.03	.97	--	.03	.05
17	10	--	1.0	--	0	0
18A	32	.03	.97	--	.06	.06
18B	250	.01	.99	--	.02	.02
19	52	.02	.98	--	.04	.04
20	136	--	1.0	--	0	0
21	180	--	.99	.01	.02	.02
22	104	.05	.94	.01	.12	.11
23	14	--	1.0	--	0	0
24	46	--	1.0	--	0	0
25	56	.05	.95	--	.11	.10
26	158	.01	.97	.02	.05	.05
27	172	.01	.99	--	.01	.01
28	176	.03	.96	.01	.06	.08
29	148	.02	.98	--	.04	.04
30	144	.01	.99	--	.01	.01
31A	220	.04	.95	.01	.09	.09
31B	318	.02	.98	--	.04	.04
31C	102	.01	.99	--	.02	.02
32	190	--	1.0	--	0	0
33	222	--	1.0	--	0	0
34	202	--	1.0	--	0	0
35	144	--	1.0	--	0	0

† No. of isofemale lines analysed given in parentheses.

TABLE 12. *Mean gene frequencies and heterozygosities.*

Locus	No. Populations Sampled	No. Wild Genomes Sampled	Gene Frequency (standard error)					% Popns. Polymorphic*		Heterozygosity	
			a	b	c	d	e	1% level	5% level	Mean	S.E.
Est-1	54	10190	.228 (.011)	.727 (.012)	.045 (.005)			100	100	.402	.013
Est-2	53	7836	.360 (.011)	.311 (.011)	.145 (.009)	.183 (.008)	.001 (.001)	100	100	.699	.004
Adh-1	50	4701	.003 (.001)	.545 (.017)	.452 (.017)			100	100	.469	.010
Adh-2	50	4701	.998 (.001)	.002 (.001)				8	0	.004	.002
Pyr	50	7568	.893 (.006)	.107 (.006)				100	92	.187	.010
Ao	53	8092	.954 (.005)	.046 (.005)				83	43	.086	.009
Pgm	52	8280	.017 (.002)	.982 (.002)	.001 (.001)			63	6	.035	.004
								Average (including monomorphic loci)		.065.	.031

* 1% level—proportion of populations where the most common allele had a frequency ≤ 0.99,
5% level—proportion of populations where the most common allele had a frequency ≤ 0.95.

same populations decreased and then increased ($b_1 = -2.910 \pm 0.825$, $P < 0.01$; $b_2 = 0.0098 \pm 0.0028$, $P < 0.01$). However, analyses with single environmental variables are not necessarily very informative, and genotype-environment associations are being analysed using multivariate techniques (Mulley, Barker and James, in preparation).

The other form of differentiation was the loss of low frequency alleles. This loss was probably an artifact of the sampling process in most cases, but in localities 1 and 2 this was considered unlikely because *all* of the usual alleles of consistent low frequency (*Est-1*^{c}, *Ao*^{b}, *Pgm*^{a}) were absent. Intensive trapping throughout one summer ensured the capture of what was probably a significant portion of the population in these small isolates. The most likely explanation appears to be true allelic extinction due to genetic drift.

Two distinct types of low frequency alleles were observed. Some were of consistent occurrence (*Est-1*^{c}, *Ao*^{b}, *Pgm*^{a}) and others were of sporadic occurrence (*Est-2*^{e}, *Adh-1*^{a}, *Pgm*^{c}, *Adh-2*^{b}). Low frequency alleles at consistent frequency throughout the distribution suggest strong selection forces maintaining the variation, as these alleles would otherwise be readily eliminated by drift. Alleles of sporadic occurrence were at such low frequency that their absence could be accountable either through the sampling process at collection or true extinction. Their widespread distribution presumably is maintained by recurrent mutation after allelic extinction through drift, or by migration.

The overall description of isozyme variation in *buzzatii* therefore agrees well with other studies of *Drosophila* species (e.g. Ayala et al., 1972; Johnson 1971; Prakash et al., 1969; Rockwood-Sluss et al., 1973; and others). The notable departure was the level of variation detected. Over 35 localities the average heterozygosity was 0.065 $\pm$ 0.031 (range 0.051–0.078) and the percentage of loci polymorphic was 15.3% (5% criterion) and 19.2% (1% criterion).

Discussion

Magnitude of Variation

The extent to which electrophoretic variation estimates absolute genetic variation depends on the extent to which electrophoretically detectable variation is representative of the genome. Possible biases were discussed by Lewontin and Hubby (1966). Kojima et al. (1970) found less variation at loci in the glucose-metabolizing system than at loci not directly involved in this system, and suggested that the greater variability in the latter might reflect greater variability in their substrates, which often originate in the external environment. G. B. Johnson (1971) suggested that the polymorphic loci often are associated with regulatory reactions in metabolism. In a later review (Johnson, 1974a), he showed that average heterozygosity was highest for variable substrate enzymes, while for enzymes utilizing specific substrates, average heterozygosity was much higher for those classed as regulatory than for those classed as non-regulatory. Thus, measures of genetic variability depend to some extent on the particular sample of loci which were chosen for study, and this must be considered in any comparisons of observed variability in different studies.

In a summary of data for 13 *Drosophila* species, Johnson (1974a) gave an average heterozygosity over all species of 0.16, with individual species ranging from 0.10 to 0.27. Ostensibly lower values of average heterozygosity have been reported for *D. busckii* —0.04 (Prakash, 1973b), for *D. mojavensis* —0.054, 0.067, and 0.082 in different races (Zouros, 1973) and now for *D. buzzatii*. For three other species of the *mulleri* subgroup, Zouros (1973) reported values that were higher than, although not significantly different from, the values for *mojavensis* and *buzzatii*. They were all, however, at the lower end of the range for *Drosophila* species, viz. 0.126 for *D. arizonensis*, 0.113 for *D. mulleri*, and 0.121 for *D. aldrichi*. A more direct comparison can be made using the eight loci common to our study and Zouros' (Table 13). Two races of *mojavensis* may be exceptional, but generally the average heterozygosity of *buzzatii* is similar to that in other species of the *mulleri* subgroup, and the average for the seven species and races is 0.136. In contrast, the average heterozygosity for loci controlling equivalent enzymes in other *Drosophila* species (extracted from Table 3 of Johnson, 1974a) is 0.198, with a range of 0.104 to 0.376. Clearly no definite conclusion can be made, but the average heterozygosity in species of the *mulleri* subgroup may be less than in other species of *Drosophila*.

TABLE 13. *Average heterozygosity ($\overline{H}$) estimated from the eight loci common to this study and that of Zouros (1973).*

Species and race	$\overline{H}$ ± standard error
mojavensis A	.023 ± .022
mojavensis B I	.133 ± .077
mojavensis B II	.086 ± .057
arizonensis	.147 ± .079
mulleri	.171 ± .093
aldrichi	.197 ± .109
buzzatii	.196 ± .100

Although *buzzatii* appears to be similar to other cactophilic *Drosophila* of the *mulleri* subgroup, the low level of heterozygosity in the Australian *buzzatii* population could be due to a founder effect associated with its introduction into Australia. Bottlenecks have been invoked to explain low levels of variation in some vertebrates (Avise and Selander, 1972; Bonnell and Selander, 1974; Webster et al., 1972). This hypothesis was tested by assaying for all 29 loci in six laboratory strains of *buzzatii* originating from Argentina, Bolivia and Lebanon. In no case did these strains possess an allele not present in Australia. On the other hand, most known alleles (*Est-2^c*, *Est-2^e*, *Adh-1^a*, *Adh-2^b* and *Pgm^c* excepted) were detected in these strains. Those not detected were at very low frequency (except for *Est-2^c*) in Australia (Tables 2 and 7–11), so that failure to detect them from a mere six strains is not

unexpected. Small samples of wild-caught *buzzatii* from South Africa and Israel (29 and 12 individuals respectively) were assayed for the seven variable loci only, and also were consistent in allelic content. *For these variable loci only*, average heterozygosities in these samples were 0.130 and 0.247, as compared with that for the Australian populations of 0.269. These results suggest that the average heterozygosity observed in Australian *buzzatii* is not the product of a founder event, but conclusive evidence will not be obtained until we are able to get large samples of wild-caught flies from South America.

The low level of genic variation in *buzzatii* could be a function of the ecology of the species, in terms either of the niche-variation hypothesis (van Valen, 1965) or of environmental grain (Levins, 1968; Gillespie, 1974). The niche-variation hypothesis postulates a positive relationship between genetic variability and niche-width. Evidence outlined above (Ecology and History) suggests that *buzzatii* has a narrow feeding and breeding niche. To this extent, the low level of genetic variation in *buzzatii* fits the niche-variation model, and is supported by apparently low variation in other cactophilic *Drosophila* (Zouros, 1973), and in *D. busckii* which supposedly has a narrow seasonal or nutritional niche (Prakash, 1973b). In contrast, species of the *willistoni* group inhabit the rain forests of South America which are ecologically both rich and diverse, and show high average heterozygosity (0.18 to 0.22—Ayala and Powell, 1972; Richmond, 1972). Comparisons among populations of the one species also could be made with regard to the niche-variation hypothesis, although the identification and measurement of significant niche dimensions poses problems. This difficulty is often resolved by equating distributionally central habitats with a rich and diversified environment and distributionally marginal habitats with a poor and uniform environment. This approach depends on the equivalence of distributional and ecological margins, which may not be correct. Further, marginal populations may be subjected to more diverse selection (temporal instability—Lewontin, 1974) than are central populations, thus tending to increase relative heterozygosity (Prakash, 1973a), rather than decrease it. On the other hand, effective population sizes in poor habitats (ecological margins) may be so low that the loss of alleles through genetic drift may reduce variability.

As has been found in comparable studies of other *Drosophila* species (Ayala et al., 1971; Prakash et al., 1969; Saura et al., 1973), the average heterozygosity showed little variation over the entire distribution. This similarity among populations could result from a relatively uniform niche throughout the distribution or from migration. Unfortunately, little is known of niche parameters or migration potential for most *Drosophila* species. For *buzzatii*, given the spatial isolation between populations and the little ecological information available, the uniform niche hypothesis appears more likely. Localities 1, 2, 20, 22, 23 and 25 are geographically peripheral isolates with small populations (as judged from collecting effort), and so, presumably, are also ecologically marginal. The slight reduction in heterozygosity at localities 1, 2 and 20 may result from low effective population size, while the slight increase in localities 22, 23, and 25 may imply more variable selection overriding any effects of low population size. However, given the standard errors of the average heterozygosities, these deviations are not significant.

The possibility that the low level of variation in *buzzatii* may be related to the graininess of its environment is an extension of the uniform niche hypothesis. Levins (1968) argued that genetic variation is less likely in fine-grained environments, and Gillespie (1974) has given analytical proof. Levins' argument was used by Selander and Kaufman (1973) to account for the lower levels of allozymic variation observed in vertebrate populations than in invertebrate populations (mainly *Drosophila*). For *Drosophila*, larval and pupal

stages are expected to experience the environment as coarse-grained. However, the environment of immature stages of *buzzatii* may be more fine-grained than that of other *Drosophila* species. The immature stages of *buzzatii* always occur in the same apparently uniform environment—in rot-pockets inside the thick, leathery covering of *Opuntia* cladodes. They are protected, at least to some extent, from external environmental variation in temperature and humidity, although there may be differences between rot-pockets or even between different parts of the one rot-pocket in temperature, pH, microflora or chemical composition.

In addition to the apparently low level of average expected heterozygosity, we find that the observed heterozygote frequency is generally less than expected. For all five variable loci where tests were possible, a number of individual populations showed significant heterozygote deficiency, and over all populations, there were significantly more cases of heterozygote deficiency than of heterozygote excess. A number of possible explanations of observed heterozygote deficiency, viz. existence of null alleles, scoring bias, selection against heterozygotes, and positive assortative mating, would seem most unlikely in this case with five loci involved. One possible explanation is the Wahlund effect where a locality sample comprises individuals from populations with different gene frequencies. These differences in gene frequency might be due simply to genetic drift resulting from the finite size of partly-isolated breeding populations, or they might be directly due to differential selection in different populations. Secondly, heterozygote deficiency could result from diversifying selection (multiple niche polymorphism) within individual populations, or even within single rot-pockets, due to a changing internal environment of a rot-pocket from its initial development as a suitable breeding site to when it finally dries up. If this pattern of environmental change was similar in all rots within a locality, similar gene frequencies could be maintained in partly-isolated breeding populations, but with an observed heterozygote deficiency. A third possible explanation involves inbreeding additional to that which would accumulate in a random mating population of finite size. This could result from the ecology and behaviour of the species, if individuals emerging in a given rot-pocket tend to remain there, thus increasing the probability of matings between relatives. Dispersal of flies as the rot-pockets dry out would prevent accumulation of this inbreeding, so that one might expect to observe heterozygote deficiency in flies emerging from some rots, but with no significant differences in gene frequency among these rots.

To determine whether inbreeding was related to subjective estimates of population size or degree of isolation of the locality, the frequency of individuals homozygous for all five variable loci (i.e. excluding *Adh*), for any four of the five, or any three, any two, any one or none of the five loci was determined for the 40 populations where wild-caught individuals were each assayed for all five loci. As the zero and one locus classes included very few individuals, they were pooled with the two locus class. The χ^2 test for differences among populations was not significant ($\chi^2_{(117)} = 128.36$), so that inbreeding would seem more likely to be a function of the ecology and behaviour species. Some effects of population size are evident in that the contributions to total χ^2 for three populations were significant. Locality 20 (significantly more homozygous than the average) is a peripheral isolate of small population size. Locality 6 (significantly less homozygous than average in collection 6D, and almost significant in collection 6E—$0.10 > P > 0.05$) probably has the largest population of all localities, with a dense infestation of *O. inermis* spread over at least 100 hectares. Locality 16B also was significantly less homozygous than average, but we would not predict a large population size for this locality.

If heterozygote deficiency were due to inbreeding, then assuming no selection, the

proportionate deficiency of heterozygotes should be the same for each locus, that is, the ratio of observed heterozygote frequency to that expected under Hardy Weinberg is a measure of (1-F). Therefore, for each of the 45 populations where either four or five loci were segregating, χ^2 was calculated to test for the significance of differences among loci in observed and expected numbers of heterozygotes. Not only were none of these χ^2 values significant, but 25 gave probabilities greater than 0.95, and all probabilities were greater than 0.3. That is, the ratios of observed to expected numbers of heterozygotes are generally significantly similar, providing further corroborative evidence for the importance of inbreeding.

Additional information is available from collections from different rots. In collection 7B, sufficient adults were aspirated directly from each of five rot pockets to warrant their separate analysis. For *Est-1*, there was a significant deficiency of heterozygotes in flies from Rot 1, and for the total collection, and gene frequencies in the different rots were significantly different; that is, results consistent with a Wahlund effect. For *Est-2*, there was an observed deficiency of heterozygotes in every rot (significant for Rot 4), and a significant deficiency of heterozygotes for the total collection, but gene frequencies in the different rots were not significantly different; that is, results consistent with either inbreeding or diversifying selection within rot pockets.

In the continuing temporal study at locality 5, rot pockets have been collected in the field on two occasions, returned to the laboratory, and all emerging adults collected for assay. In March, 1974, sufficient flies were collected from four such rots. For both *Est-1* and *Est-2*, there was a significant deficiency of heterozygotes in the total collection from all rots, but gene frequencies were significantly different among rots only for *Est-2*. In the second case, (July, 1974—again four rots), heterozygotes were not significantly deficient for any locus, and there were no significant differences among rots in gene frequencies at any locus. The difference between these two rot collections is consistent with other data from the temporal study, and leads us to postulate some important aspects of the ecology and behaviour of the species. In the temporal study, collections are being made each month at 10 defined sites within the *O. inermis* infestation. In the first year of the study (to January, 1975) significant differences in gene frequencies among sites have been found for all five loci, but only in the period November to May, and with 13 of 15 such cases in the period November to March. This coincides with the period of *Cactoblastis* activity when new rots are continually developing. Our field observations indicate that these new rots in the fleshy cladodes may remain as suitable breeding sites for at least four to six weeks. Following colonization, two generations could develop in each rot, and we postulate that at this time, individual flies emerging in a particular rot tend to remain there, the founder event and subsequent inbreeding leading to genetic differences among rot pockets and a deficiency of heterozygotes. From late summer to autumn, these rots dry out, and the population becomes dependent for overwintering on the much smaller number of old, long-persistent rots in the basal "stems" of the *Opuntia*. The flies inhabiting these rots from autumn through winter will include not only any that developed there in the previous summer, but also a high proportion of migrants from surrounding areas. Therefore gene frequencies would not be expected to be different among sites, although inbreeding still could accumulate during the winter months.

Maintenance of Variation

The existence of extensive genic variation in natural populations is now generally accepted. Irrespective of whether gene frequencies are low or at intermediate levels the variation is generally stable in space and time. This observation has prompted support for various models of balancing natural selection. Further,

studies where selection has been directly or indirectly implicated argue against the hypothesis of neutral variation (Johnson et al., 1969; Koehn and Rasmussen, 1967; Rockwood-Sluss et al., 1973; Taylor and Mitton, 1974; Tomaszewski et al., 1973).

Johnson (1974b) has argued that the two models proposed for estimation of the effective number of selectively neutral alleles maintained in a finite population, viz. $n_e = 1 + 4N_e\mu$ (Kimura and Crow, 1964), and $n_e = \sqrt{1 + 8N_e\mu}$ (Ohta and Kimura, 1973), define the limiting cases. Using these models, we have estimated N_e by substituting observed values of n_e (the reciprocal of sums of squares of allele frequencies) and assuming mutation rates of 10^{-5} to 10^{-7} (Mukai, 1970; Tobari and Kojima, 1972). For localities 1 and 2, the estimated N_e ranges from 1300 to 150,000. The sparsity of *Opuntia* and the very low catch per unit effort during the most favourable period of the year suggests that the upper limit, and probably even the lower limit, is grossly unrealistic. Therefore, either some form of balancing selection is operating or these localities are not genetically isolated. The latter possibility seems unlikely because, if there were migration into these localities, they should contain the alleles *Est-1*^{c}, *Pgm*^{a} and *Ao*^{b} that occur at low frequency throughout the rest of the species distribution. But these alleles were all absent; probably lost by drift. It may be possible to miss at least one of these alleles at sampling, but all three should not have escaped detection, because the samples from both localities probably represented a significant proportion of the population.

Tests of selective neutrality are beset with problems arising from a scarcity of ecological data on *Drosophila*. Migration rates, effective population sizes, selection intensities and even mutation rates to neutral alleles are not accurately known.

However, selective neutrality as a realistic model relies ultimately on migration between populations (Kimura and Ohta, 1971) and results from localities 1 and 2 indicate that these populations are probably genetically isolated. This observation, and the occurrence of a number of low frequency alleles at consistent frequency throughout the species distribution, suggest that some form of balancing selection is operating.

Summary

Drosophila buzzatii is a cactophilic species of the *mulleri* subgroup. Genetic variation was measured for 29 loci in 50 populations from 35 localities throughout the structured and potentially known distribution in eastern Australia. The average population was polymorphic for 15.3% (5% criterion) and 19.2% (1% criterion) of the loci sampled. The average individual was heterozygous at only 6.5% of the loci sampled. This low level of heterozygosity is considered in terms of founder effect, niche width and environmental grain, and it is suggested that the cactophilic species of the *mulleri* subgroup may show less genic variation than other *Drosophila* species. The low level of genetic variation appears not to be due to a founder effect associated with the species' introduction into Australia. For the five variable loci where tests were possible, heterozygote frequencies were generally less than expected, possibly due to the combined or individual effects of Wahlund's principle, extra inbreeding above that due to finite population size because of the mating of close relatives and diversifying selection. At least two of the localities sampled appear to be genetically isolated and it is argued that some form of balancing selection is operating to maintain variation.

Acknowledgments

We are grateful for the invaluable cooperation generously given by the officers of the Prickly Pear Destruction Commission in New South Wales and the Lands Department in Queensland, and the farmers and graziers who have allowed us access to their properties and assisted in location of cactus infestations. Dr. I. Franklin assisted with technical advice on electrophoretic proce-

dures in the initial stages of the research, and Dr. F. W. Nicholas stimulated our thinking on effective inbreeding. We are indebted to Dr. M. R. Wheeler for supplying the laboratory strains from the University of Texas collection, and to Mr. A. L. Dyce and Dr. R. Falk for the samples of wild-caught flies from South Africa and Israel respectively. The research was supported by the Australian Research Grants Committee.

Literature Cited

Avise, J. C., and R. K. Selander. 1972. Evolutionary genetics of cave-dwelling fishes of the genus *Astyanax*. Evolution 26:1–19.

Ayala, F. J., and J. R. Powell. 1972. Enzyme variability in the *Drosophila willistoni* group. VI. Levels of polymorphism and the physiological function of enzymes. Biochem. Genet. 7:331–345.

Ayala, F. J., J. R. Powell, and Th. Dobzhansky. 1971. Polymorphisms in continental and island populations of *Drosophila willistoni*. Proc. Nat. Acad. Sci. 68:2480–2483.

Ayala, F. J., J. R. Powell, M. L. Tracey, C. A. Mourão, and S. Pérez-Salas 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genetic variation in natural populations of *Drosophila willistoni*. Genetics 70:113–139.

Bonnell, M. L., and R. K. Selander. 1974. Elephant seals: genetic variation and near extinction. Science 184:908–910.

Bryant, E. H. 1974*a*. On the adaptive significance of enzyme polymorphisms in relation to environmental variability. Amer. Natur. 108: 1–19.

——. 1974*b*. An addendum on the statistical relationship between enzyme polymorphisms and environmental variability. Amer. Natur. 108: 698–701.

Carson, H. L., and M. Wasserman. 1965. A widespread chromosomal polymorphism in a widespread species, *Drosophila buzzatii*. Amer. Natur. 99:111–115.

Gillespie, J. 1974. The role of environmental grain in the maintenance of genetic variation. Amer. Natur. 108:831–836.

Hubby, J. L., and R. C. Lewontin. 1966. A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. Genetics 54:577–594.

Johnson, F. M. 1971. Isozyme polymorphisms in *Drosophila ananassae*: genetic diversity among island populations in the South Pacific. Genetics 68:77–95.

Johnson, F. M., C. G. Kanapi, R. H. Richardson, M. R. Wheeler, and W. S. Stone. 1966. An analysis of polymorphisms among isozyme loci in dark and light *Drosophila ananassae* strains from American and Western Samoa. Proc. Nat. Acad. Sci. 56:119–125.

Johnson, F. M., and H. E. Schaffer. 1973. Isozyme variability in species of the genus *Drosophila*. VII. Genotype-environment relationships in populations of *D. melanogaster* from the eastern United States. Biochem. Genet. 10: 149–163.

Johnson, F. M., H. E. Schaffer, J. E. Gillaspy, and E. S. Rockwood. 1969. Isozyme genotype-environment relationships in natural populations of the harvester ant, *Pogonomyrmex barbatus*, from Texas. Biochem. Genet. 3: 429–450.

Johnson, G. B. 1971. Metabolic implications of polymorphism as an adaptive strategy. Nature 232:347–349.

——. 1974a. Enzyme polymorphism and metabolism. Science 184:28–37.

——. 1974b. On the estimation of effective number of alleles from electrophoretic data. Genetics 78:771–776.

Kimura, M., and J. Crow. 1964. The number of alleles than can be maintained in a finite population. Genetics 49:725–738.

Kimura, M., and T. Ohta. 1971. Protein polymorphism as a phase of molecular evolution. Nature 229:467–469.

Koehn, R. K., and D. J. Rasmussen. 1967. Polymorphic and monomorphic serum esterase heterogeneity in catastomid fish populations. Biochem. Genet. 1:131–144.

Kojima, K., J. Gillespie, and Y. N. Tobari. 1970. A profile of *Drosophila* species' enzymes assayed by electrophoresis. I. Number of alleles, heterozygosities, and linkage disequilibrium in glucose-metabolising systems and some other enzymes. Biochem. Genet. 4:627–637.

Kojima, K., P. Smouse, S. Yang, P. S. Nair, and D. Brncic. 1972. Isozyme frequency patterns in *Drosophila pavani* associated with geographical and seasonal variables. Genetics 72:721–731.

Levins, R. 1968. Evolution in Changing Environments, Princeton Univ. Press, New Jersey.

Lewontin, R. C. 1974. The Genetic Basis of Evolutionary Change, Columbia University Press, New York.

Lewontin, R. C., and J. L. Hubby. 1966. A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics 54:595–609.

Mann, J. 1970. Cacti naturalised in Australia and their control. S. G. Reid, Government Printer, Brisbane.

MUKAI, T. 1970. Spontaneous mutation rates of isozyme genes in *Drosophila melanogaster*. Drosophila Inform. Serv. 45:99.

MULLEY, J. C. 1973a. Successive enzyme staining on acrylamide gels. Drosophila Inf. Serv. 50: 201.

——. 1973b. Electrophoretic detection of pyranosidase in *Drosophila buzzatii*. Drosophila Inf. Serv. 50:139.

OHTA, T., AND M. KIMURA. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genet. Res. 22:201–204.

PRAKASH, S. 1973a. Patterns of gene variation in central and marginal populations of *Drosophila robusta*. Genetics 75:347–369.

——. 1973b. Low gene variation in *Drosophila busckii*. Genetics 75:571–576.

PRAKASH, S., R. C. LEWONTIN, AND J. L. HUBBY. 1969. A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of *Drosophila pseudoobscura*. Genetics 61:841–858.

RICHMOND, R. C. 1972. Enzyme variability in the *Drosophila willistoni* group. III. Amounts of variability in the superspecies, *D. paulistorum*. Genetics 70:87–112.

ROCKWOOD-SLUSS, E. S., J. S. JOHNSTON, AND W. B. HEED. 1973. Allozyme genotype-environment relationships. I. Variation in natural populations of *Drosophila pachea*. Genetics 73: 135–146.

SAURA, A., S. LAKOVAARA, J. LOKKI, AND P. LANKINEN. 1973. Genic variation in central and marginal populations of *Drosophila subobscura*. Hereditas 75:33–46.

SELANDER, R. K., AND W. E. JOHNSON. 1973. Genetic variation among vertebrate species. Ann. Rev. Ecol. Systematics 4:75–91.

SELANDER, R. K., AND D. W. KAUFMAN. 1973. Genic variability and strategies of adaptation in animals. Proc. Nat. Acad. Sci. 70:1875–1877.

TAYLOR, C. E., AND J. B. MITTON. 1974. Multivariate analysis of genetic variation. Genetics 76:575–585.

TOBARI, Y. N., AND K. KOJIMA. 1972. A study of spontaneous mutation rates at ten loci detectable by starch gel electrophoresis in *Drosophila melanogaster*. Genetics 70:397–403.

TOMASZEWSKI, E. K., H. E. SCHAFFER, AND F. M. JOHNSON. 1973. Isozyme genotype-environment associations in natural populations of the harvester ant, *Pogonomyrmex badius*. Genetics 75:405–421.

VAN VALEN, L. 1965. Morphological variation and width of ecological niche. Amer. Natur. 99:377–390.

WASSERMAN, M. 1954. Cytological studies of the *repleta* group. Univ. Texas Publ. 5422:130–152.

WEBSTER, T. P., R. K. SELANDER, AND S. Y. YANG. 1972. Genetic variability and similarity in the *Anolis* lizards of Bimini. Evolution 26:523–535.

ZOUROS, E. 1973. Genetic differentiation associated with the early stages of speciation in the *mulleri* subgroup of *Drosophila*. Evolution 27: 601–621.