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Breeding structure of natural populations of *Drosophila buzzatii*: effects of the distribution of larval substrates

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The population structure of several Australian populations of the cactophilic *Drosophila buzzatii* was investigated, with seasonal samples from two populations analyzed for six polymorphic allozymes. Significant inbreeding throughout the year was detected in both populations, and significant but relatively slight differences in allele frequencies between collections were found in one population. Little significant 2-locus linkage disequilibrium was detected and the variances of linkage disequilibrium coefficients were generally consistent with genetic drift. Individual breeding substrates (rotting cactus cladodes) were collected and the adult flies emerging from them were scored for their allozyme genotypes. The data suggest that approximately ten individuals contribute to the progeny emerging from a rot. The influence of the sizes and distributions of breeding substrates on the maintenance of genetic variation is discussed.

INTRODUCTION

An understanding of the breeding structure of populations is essential for explaining the maintenance of genetic variation, and changes in gene and genotype frequencies over space and time. Breeding structure is the base-line to which various scales of spatial and temporal environmental variation must be related to evaluate their significance in evolution.

Drosophila species have been the subject of many laboratory studies on the effects of spatial and temporal variation on genetic variation (*e.g.*, McDonald and Ayala, 1974; Powell, 1971; Powell and Wistrand, 1978), but most species are not suitable for studies of breeding structure in natural populations because of our general ignorance about their ecology. Members of the *mulleri* subgroup of the *repleta* group of *Drosophila* are a conspicuous exception, because most of them feed and breed exclusively in decaying cactus tissue, often with considerable host specificity (Heed, 1978; Heed, 1982; Heed and Mangan, 1986). One member of the subgroup, *D. buzzatii*, has spread from South America around the world together with its host species of *Opuntia* cactus (Barker and Mulley, 1976). It has a widespread distribution in eastern Australia and provides a powerful model system for studies in ecological genetics (*e.g.*, Barker *et al.*, 1986; Sokal *et al.*, 1987). A long term study of polymorphic allozymes within a single population suggested that microspatial heterogeneity at the level of individual breeding substrates (rots) is very important in maintaining polymorphismns (Barker *et al.*, 1986).

In order to understand the breeding structure of *D. buzzatii*, we investigated the allozyme genotypes of flies emerging from particular rots, and compared them to samples of the population at large. Measures of inbreeding and linkage disequilibrium were calculated, to throw light on breeding structure and to estimate the effective number of parents contributing to the flies emerging from a rot. These measures relate the pattern of matings in a population to breeding substrates, and suggest mechanisms that could maintain the polymorphisms.

METHODS

Seasonal collections were made at bi-monthly intervals throughout 1986 at two sites in New South

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Wales. These sites are O'Hara (30°26′S, 150°39′E), in a paddock in the Hunter River Valley near Denman, and Trinkey (31°22′S, 149°27′E), located near Tambar Springs, in a transition from paddock to forest edge, approximately 150 km from O'Hara. Flies were collected from fermenting banana baits, by net or by aspiration. Twenty to thirty baits were spread widely through the habitat and were checked in a circuit until enough flies were obtained or until collecting conditions deteriorated.

Collections from individual rotting cactus cladodes (rots) were made on two occasions from Trinkey, once from O'Hara, and once from each of four sites in southeast Queensland (near Brisbane) viz.: (1) Grandchester (27°40′S, 152°28′E), (2) Grandchester Hill (27°40′S, 152°28′E), (3) Borallon (27°31′S, 152°43′E), and (4) Hemmant (27°27′S, 153°3′E). Individual rots were maintained in gauze covered containers on moist sand held at 25°C and emerging adults were aspirated from them daily.

Six enzyme loci known to be polymorphic in these populations were assayed using the methods of Barker and Mulley (1976) and Barker, East and Weir (1986). Knibb *et al.* (1987) may be consulted for further information on the scoring of esterase alleles. The loci are *esterase*-1 (*Est*-1), *esterase*-2 (*Est*-2), *β-N-acetyl-hexosaminidase* (*Hex*), *phosphoglucomutase* (*Pgm*), *aldehyde oxidase* (*Aldox*), and *alcohol dehydrogenase*-1 (*Adh*-1). All these loci are autosomal. *Est*-1, *Est*-2 and *Aldox* are on chromosome II, with *Est*-1 and *Aldox* being within a polymorphic inversion (2j) and *Est*-2 just beyond the inversion breakpoint (Schafer, personal communication). In the Standard arrangement (2st), *Aldox* maps between *Est*-1 and *Est*-2 14 cM from *Est*-1 and 21 cM from *Est*-2. The j arrangement order is *Aldox*, *Est*-1, *Est*-2 with *Est*-1 37 cM from *Est*-2 (Schafer, personal communication). *Adh*-1 and *Hex* are on chromosome III but show no linkage. *Pgm* is on chromosome IV.

Statistical analyses

Allelic frequencies and F-statistics

Analyses of allelic frequencies and the calculation of *F*-statistics using the methods of Weir and Cockerham (1984) were accomplished with the program GENSTATS, kindly made available by Krafsur (Black and Krafsur, 1985a). *F*-statistics were also calculated by Nei's (1977) methods. The use of either method did not alter the qualitative conclusions presented here.

Tests of selective neutrality were performed by the method of Lewontin and Krakauer (1973), where significant heterogeneity among loci in a measure of genetic variation indicates selective differences. This test is based on the idea that genetic drift will affect all loci similarly while selection will affect different regions of the genome differently. Some of the limitations of this test will be discussed later. The measure of genetic variation used here was F_{ST} and the significance test compared the ratio of the observed variance in F_{ST} over its expected variance to an $F_{(n-1,\cdot)}$ distribution, where n was the number of sampling periods and the dot indicates an average taken over all alleles at a locus. The observed variance was calculated as,

$$s_F^2 = \sum_{j=1}^{m} \frac{N_j}{N} (\hat{F}_{ST.j} - \bar{F}_{ST})^2, \qquad (1)$$

where N is the total sample size and N_j is the sample size of the jth locus considered over m loci. The expected variance is,

$$\sigma_F^2 = \frac{k\bar{F}_{ST}^2}{(n-1)} \qquad (2)$$

where the parameter k is set equal to 2 (Lewontin and Krakauer, 1973) and n is the number of sampling periods.

Linkage disequilibria

Analyses of linkage disequilibria were done on a two-allele collapse of the data, so as to eliminate problems due to very small numbers in some genotypic classes. The most common alleles at multiallelic loci were retained and all the others pooled. Diallelic two-locus disequilibrium coefficients were calculated with Cockerham and Weir's (1977) composite measure,

$$\Delta_{ij} = D_w^{ij} + D_b^{ij},$$

where D_w^{ij} is the within-gamete disequilibrium component between allele i at locus A and allele j at locus B. Similarly, D_b^{ij} is the between-gamete disequilibrium component for the same alleles. Δ_{ij} has the desirable properties of being unbiased with respect to departures from random mating and is usable with genotypic data rather than gametic or haplotype data (Cockerham and Weir, 1977). Δ_{ij}, when normalized by the products of allele frequencies as,

$$r_{ij} = \Delta_{ij} / [(p_i(1-p_i) + D_i^i)(p_j(1-p_j) + D_j^j)]^{1/2}$$

gives the correlation of allele frequencies between the two loci (Weir, 1979). The D_i^i and D_j^j are measures of departure from Hardy-Weinberg equilibrium at each locus. Tests of the hypothesis that $\Delta_{ij} = 0$ were done with the statistic,

$$X^2 = N(r_{ij}^2),$$

where N is the number of individuals sampled. X^2 is distributed approximately as χ^2 with one degree of freedom when considering particular pairs of alleles. For calculating disequilibria between loci the statistic is summed over two-allele interactions as,

$$X^2 = N \sum_i \sum_j (\Delta_{ij}^2 / p_i p_j),$$

which is also distributed approximately as χ^2 with $(m-1)(n-1)$ degrees of freedom, where m and n are the number of alleles at loci A and B respectively (Weir, 1979). Using these tests with the present data, each has one degree of freedom.

The effects of population subdivision on linkage disequilibria were analyzed under the model of Ohta (1982*b*) which incorporates a finite island model with extinction and replacement of colonies. Her model partitions the variance of linkage disequilibrium into components in a manner roughly analogous to Wright's partitioning of the inbreeding coefficient using F-statistics (Wright, 1969). By comparing five measures of the variance of disequilibrium describing total, within- and between-subdivision components, Ohta's method (1982*a*) is able to distinguish between drift due to limited dispersal and epistatic natural selection. These five measures of variance of disequilibrium are: (1) D_{IT}^2, total variance of disequilibrium, (2) D_{IS}^2, variance of within-subpopulation disequilibrium, (3) D_{ST}^2, variance of the correlation of the ith alleles of loci A and B of different gametes of one subpopulation relative to the total population, (4) $D_{IS}'^2$, variance of the correlation between the ith alleles of loci A and B of one gamete of a subpopulation relative to that of the average gamete of the population, and (5) $D_{ST}'^2$, variance of the ordinary disequilibrium of the whole population. Three of these measures are related as,

$$D_{IT}^2 = D_{IS}'^2 + D_{ST}'^2.$$

When genetic drift resulting from limited migration is primarily responsible for the observed disequilibrium $D_{IS}^2 < D_{ST}^2$ and $D_{IS}'^2 > D_{ST}'^2$, because the variation among populations is expected to exceed that within populations. When epistatic selection is important for linkage disequilibrium but not for local differentiation, $D_{IS}^2 > D_{ST}^2$ and $D_{IS}'^2 < D_{ST}'^2$. This is simply because gametes with favourable combinations of alleles should increase in all colonies. When selection acts, but not systematically, *i.e.*, not in the same direction in each subpopulation, $D_{IS}^2 > D_{ST}^2$ and $D_{IS}'^2 > D_{ST}'^2$. Ohta (1982*a*, *b*) should be consulted for the derivations of these relationships.

Analyses of linkage disequilibria, and of the effects of population subdivision on the variance of linkage disequilibrium coefficients, were carried out using the program LINKDIS (Black and Krafsur, 1985*b*), modified to accommodate larger data sets.

RESULTS

Data from the seasonal collections, which should represent random samples of the populations from which they were drawn, will be considered first. These samples are the baseline with which to compare the samples emerging from rots, considered in the following section.

Seasonal collections

Table 1 gives the allele frequencies observed at each locus for each collection. Table 2 shows the alleles with significant frequency differences among collections within sites. Since fewer collections were obtained from Trinkey, it might be thought that the lack of significant temporal variation in the frequencies of common alleles was simply the result of sampling too short a time span. However, when only those collections at directly comparable times from both locations were analyzed, most of the significant variation in allele frequencies at O'Hara remained. Variation in allele frequencies is thus greater in the O'Hara population than in the Trinkey population.

As a conservative test of departures from random mating, chi-square tests for the homogeneity of observed and expected heterozygosities for each locus within a collection were performed. Table 2 gives the proportions of tests which showed significant heterozygote excess or deficiency. Only significant deficiencies were found. Of the possible explanations for this result, inbreeding and the Wahlund effect are the most likely. Seasonal collections in a sense are pooled samples from individual breeding substrates, and the sampling scheme should have included many such substrates. It is therefore difficult to disentangle the effects of inbreeding over the entire population and the Wahlund effect due to the pooling of subdivisions.

Table 1 Allele frequencies from seasonal collections at two sites (O'Hara and Trinkey) in NSW, with sample sizes (N)

	O'Hara					Trinkey			
Locus	Apr. 86	June 86	Aug. 86	Oct. 86	Dec. 86	Aug. 86	Oct. 86	Nov. 86	Dec. 86
Pgm									
(N)	99	183	126	196	187	197	193	190	178
a	0·030	0·033	0·008	0·018	0·013	0·061	0·054	0·029	0·056
b	0·970	0·956	0·992	0·980	0·987	0·939	0·943	0·968	0·944
c	0·000	0·011	0·000	0·003	0·000	0·000	0·003	0·003	0·000
Aldox									
(N)	95	184	123	186	175	194	185	159	195
a*	0·179	0·090	0·163	0·102	0·137	0·095	0·105	0·075	0·085
a	0·716	0·793	0·736	0·774	0·743	0·763	0·749	0·802	0·764
b*	0·005	0·002	0·028	0·038	0·049	0·052	0·041	0·031	0·077
b	0·063	0·068	0·069	0·078	0·069	0·075	0·095	0·082	0·064
c	0·037	0·027	0·004	0·008	0·003	0·015	0·011	0·009	0·010
Hex									
(N)	99	184	122	192	186	196	191	185	198
a*	0·000	0·011	0·000	0·000	0·000	0·010	0·005	0·000	0·010
a	0·747	0·758	0·750	0·786	0·737	0·773	0·788	0·792	0·720
b	0·253	0·231	0·250	0·214	0·263	0·217	0·207	0·208	0·270
Adh-1									
(N)	80	175	123	170	104	187	177	152	172
b	0·463	0·491	0·528	0·544	0·668	0·487	0·551	0·549	0·503
c	0·538	0·509	0·472	0·456	0·332	0·513	0·449	0·451	0·497
Est-1									
(N)	101	184	126	191	187	197	192	187	198
a	0·109	0·141	0·096	0·134	0·115	0·190	0·203	0·195	0·217
x	0·000	0·014	0·016	0·008	0·032	0·018	0·010	0·021	0·051
b	0·743	0·813	0·857	0·819	0·834	0·749	0·766	0·751	0·687
b^-	0·084	0·014	0·008	0·000	0·011				
c	0·059	0·019	0·024	0·039	0·008	0·033	0·021	0·032	0·045
d	0·005	0·000	0·000	0·000	0·000				
Est-2									
(N)	101	184	122	186	182	197	184	175	190
a	0·342	0·359	0·254	0·336	0·360	0·355	0·394	0·426	0·368
b	0·277	0·274	0·553	0·379	0·409	0·396	0·367	0·343	0·361
c	0·094	0·114	0·098	0·113	0·060	0·099	0·106	0·106	0·111
c^+	0·025	0·046	0·004	0·003	0·003	0·008	0·008	0·023	0·005
c^f	0·020	0·008	0·008	0·008	0·014	0·018	0·003	0·000	0·016
d	0·243	0·196	0·082	0·159	0·148	0·119	0·120	0·089	0·134
e	0·000	0·003	0·000	0·003	0·005	0·005	0·003	0·014	0·005

Superscripts on allele designations indicate mobility variants discovered after the non-superscripted alleles had all been named.

Table 3 presents F-statistics calculated by the methods of Weir and Cockerham (1984). Both sites exhibited substantial inbreeding over all collections, which was also apparent in individual collections. Differentiation among collections was seen in F_{ST} values. Trinkey showed very slight differentiation while O'Hara showed more, though still relatively low, differentiation.

Selective differences among loci were tested using Lewontin and Krakauer's (1973) method. There are three assumptions made when applying this test to temporal data (Gaines and Whittam, 1980). First, gene frequency estimates in each sampling period must be based on independent samples. Since samples were taken at intervals of at least two months, different generations were sampled, and so this assumption is met. Secondly, gene frequency distributions in different populations must be identical and unimodal. The observed heterogeneity of allele frequencies at O'Hara violates this assumption. However, this results in the parameter k (equation (1)) being less than two, which biases the test against the rejection of selective neutrality. Finally, Lewontin and Krakauer (1973) state that the number of independent observations for each $\hat{F}_{ST}$ must be greater

Table 2 Allozyme alleles showing significant frequency differences ($P < 0{\cdot}05$) between collections within sites. Square brackets indicate alleles present at frequencies less than 5 per cent. Proportion of seasonal collections from O'Hara and Trinkey showing a significant excess or deficiency of heterozygotes compared to that expected under Hardy-Weinberg proportions ($N = 9$, $P < 0{\cdot}05$)

	Allele		Proportion showing significant	
Locus	O'Hara	Trinkey	Deficiency	Excess
Pgm	b, [c]		0·11	0
Aldox	a*, [b*, c]	[b*]	0·22	0
Hex	[a*]		0·22	0
Adh-1	b, c		0·11	0
Est-1	b, [c, x, b^-]	[x]	0·67	0
Est-2	b, d, [c^+]	[c^f]	0·56	0

than eight to detect heterogeneity. O'Hara was sampled five times and Trinkey four times. Trinkey, not surprisingly, showed no heterogeneity among loci ($P > 0{\cdot}05$). O'Hara, on the other hand, did show significant heterogeneity among loci ($P < 0{\cdot}05$), suggesting that the allozyme loci, or closely linked regions, are under different selective regimes.

Two-locus linkage disequilibria on a 2-allele collapse of the seasonal data showed few significant values (some were expected by chance) and these had no discernible pattern. Sample sizes within collections were too small to allow much power in these tests, but the results are not surprising given the common observation of very little observed linkage disequilibrium in natural populations of *Drosophila* (Langley *et al.*, 1978; Laurie-Ahlberg and Weir, 1979). However, Barker, East and Weir (1986) found significant disequilibrium between *Est*-2 and *Aldox*, and *Hex* and *Aldox* in *D. buzzatii*. Examination of variances of linkage disequilibrium coefficients under Ohta's model showed some differences between the populations. At O'Hara the values for all pairs of loci were consistent with drift ($D^2_{IS} < D^2_{ST}$ and $D'^2_{IS} > D'^2_{ST}$), while at Trinkey the following pairs suggest selection acting in a nonsystematic manner across loci ($D^2_{IS} > D^2_{ST}$ and $D'^2_{IS} > D'^2_{ST}$): *Aldox* and *Est*-1, *Aldox* and *Est*-2, *Adh*-1 and *Est*-2, and *Est*-1 and *Est*-2. The remaining pairs of Trinkey loci were consistent with drift. Table 4 lists these variance components for Trinkey collections.

Rot collections

All collections from all locations showed significant ($P < 0{\cdot}05$) heterogeneity of allele frequencies among rots, probably due to small numbers of "founders". This conclusion was bolstered by the general deficiency of heterozygotes (table 5), except for *Adh*-1, where there was a tendency towards heterozygote excess.

Tables 6–11 present summary *F*-statistics for each collection of rots. F_{IT} values were significant at all sites and were larger than the seasonal F_{IT}'s, implying either a nonrandom sample of the population relative to the baited seasonal samples, or the presence of sib-groups in rot emergences (*i.e.*, $N_e \ll N_0$, where N_0 is the census population size). The latter possibility is more likely given the sizes of some collections. F_{ST} values were significant at all sites and were generally three to ten times those for seasonal collections, indicating substantial differentiation among rots (coancestry within rots).

Analysis of 2-locus linkage disequilibrium coefficients and their partitioned variances revealed interesting patterns. Three pairs of loci, *Aldox* and *Est*-1, *Est*-1 and *Est*-2, and *Hex* and *Adh*-1, showed significant disequilibria considered at the level of rots (table 12). All of these pairs have both members located on the same chromosome, thus this result was probably due simply to

Table 3 *F*-statistics on four seasonal collections from Trinkey, NSW and five seasonal collections from O'Hara, NSW

	F_{IS}		F_{ST}		F_{IT}		N	
Locus	O'Hara	Trinkey	O'Hara	Trinkey	O'Hara	Trinkey	O'Hara	Trinkey
Pgm	0·143	0·000	0·004	0·001	0·146	0·002	791	754
Aldox	0·042	0·075	0·003	0·000	0·045	0·075	763	730
Hex	0·066	0·081	−0·001	0·002	0·065	0·083	783	766
Adh-1	0·095	−0·083	0·016	0·002	0·109	−0·081	652	687
Est-1	0·191	0·170	0·003	0·001	0·194	0·171	789	770
Est-2	0·037	0·214	0·020	0·000	0·057	0·214	775	742
Mean	0·077*	0·096*	0·011**	0·001**	0·087***	0·097*		

*, ** and *** indicate statistical difference from zero at probability levels of 0·05, 0·01 and 0·001 respectively.

Table 4 Variance components of linkage disequilibrium coefficients for Trinkey seasonal collections

Loci compared	Within subpopulation components		Between subpopulation components		Total population component
	D^2_{IS}	D'^2_{IS}	D^2_{ST}	D'^2_{ST}	D^2_{IT}
Pgm & Aldox	0·00049	0·00489	0·00111	0·00003	0·00492
Pgm & Hex	0·00008	0·00784	0·00181	0·00000	0·00785
Pgm & Adh-1	0·00025	0·00550	0·00159	0·00015	0·00565
Pgm & Est-1	0·00004	0·00526	0·00187	0·00002	0·00528
Pgm & Est-2	0·00035	0·00532	0·00135	0·00002	0·00534
Aldox & Hex	0·00086	0·00606	0·00168	0·00075	0·00681
Aldox & Adh-1	0·00039	0·00928	0·00143	0·00010	0·00938
Aldox & Est-1	0·00180	0·00526	0·00167	0·00168	0·00694
Aldox & Est-2	0·00202	0·00455	0·00127	0·00044	0·00500
Hex & Adh-1	0·00042	0·00794	0·00190	0·00012	0·00806
Hex & Est-1	0·00095	0·00947	0·00254	0·00000	0·00947
Hex & Est-2	0·00019	0·00662	0·00172	0·00001	0·00664
Adh-1 & Est-1	0·00177	0·00674	0·00193	0·00000	0·00674
Adh-1 & Est-2	0·00257	0·00839	0·00156	0·00000	0·00838
Est-1 & Est-2	0·00289	0·00844	0·00180	0·00290	0·01134

Table 5 Proportion of rots at four sites in Queensland and at O'Hara and Trinkey in NSW showing a significant excess or deficiency of heterozygotes compared to that expected under Hardy-Weinberg ($N = 95$, $P < 0·05$)

Locus	Proportion showing	
	Deficiency	Excess
Pgm	0	0
Aldox	0·053	0
Hex	0·084	0
Adh-1	0·021	0·042
Est-1	0·189	0
Est-2	0·263	0·011

the relatedness of flies emerging from a rot. The partitioned variances of linkage disequilibrium coefficients were consistent with drift ($D^2_{IS} < D^2_{ST}$ and $D'^2_{IS} > D'^2_{ST}$) for all pairs of loci and at all sites (tables 13 and 14 give these results for Trinkey and O'Hara, respectively. All four Queensland sites yield very similar results.). Total variance (D^2_{IT}) for rots was 10 to 20 times that of seasonal collections—another reflection of small effective population size within rots.

DISCUSSION

The breeding structure of *D. buzzatii* populations is strongly affected by their discrete and somewhat ephemeral feeding and breeding substrates. In the Australian populations studied, population size is controlled in part by seasonal variation in temperature and in part by the bivoltine life-cycle of the moth *Cactoblastis cactorum* (Murray, 1982), which generates substantial variation in the amount of rotting cactus tissue available (personal observation). The longevity of a rot, which influen-

Table 6 *F*-statistics on adult flies emerging from rots collected at Trinkey, NSW in June 1986 ($N = 4$) and in December 1986 ($N = 5$). Only rots yielding at least ten individuals were included in the analysis

Locus	F_{IS}		F_{ST}		F_{IT}		F	
	June '86	Dec. '87	June '86	Dec. '87	June '86	Dec. '87	June '86	Dec. '87
Pgm	−0·031	−0·052	0·018	0·014	−0·012	−0·037	179	219
Aldox	0·096	0·061	0·054	0·012	0·145	0·072	156	209
Hex	0·226	0·138	0·018	0·040	0·240	0·172	158	208
Adh-1	−0·001	0·125	0·011	0·062	0·009	0·179	170	210
Est-1	0·063	0·191	0·147	−0·002	0·201	0·190	176	213
Est-2	0·129	0·099	0·068	0·015	0·188	0·112	177	206
Mean	0·090*	0·115	0·059**	0·025**	0·144**	0·137***		

*, ** and *** indicate statistical difference from zero at probability levels of 0·05, 0·01 and 0·001 respectively.

Table 7 *F*-statistics on adult flies emerging from seven rots at O'Hara, NSW in June 1986. Only rots yielding at least ten individuals were included in the analysis

Locus	F_{IS}	F_{ST}	F_{IT}	*N*
Pgm	0·210	−0·008	0·204	348
Aldox	0·086	−0·001	0·085	254
Hex	0·279	0·002	0·281	339
Adh-1	0·019	0·002	0·020	329
Est-1	0·319	−0·001	0·318	341
Est-2	0·088	0·004	0·092	344
Mean	0·143	0·002*	0·144**	

* and ** indicate statistical difference from zero at probability levels of 0·05 and 0·01 respectively.

Table 8 *F*-statistics on adult flies emerging from 26 rots at Grandchester, Queensland in November 1978. Only rots yielding at least ten individuals were included in the analysis

Locus	F_{IS}	F_{ST}	F_{IT}	*N*
Pgm	0·005	0·058	0·063	578
Aldox	0·061	0·051	0·109	578
Hex	−0·095	0·024	−0·069	577
Adh-1	−0·093	0·036	−0·054	577
Est-1	0·067	0·039	0·104	578
Est-2	0·254	0·041	0·285	576
Mean	0·070	0·038***	0·106*	

* and *** indicate statistical difference from zero at probability levels of 0·05 and 0·001 respectively.

Table 9 *F*-statistics on adult flies emerging from five rots at Grandchester Hill, Queensland in November 1978. Only rots yielding at least ten individuals were included in the analysis

Locus	F_{IS}	F_{ST}	F_{IT}	*N*
Pgm	0·000	−0·024	−0·025	104
Aldox	−0·046	0·031	−0·014	104
Hex	−0·230	0·091	−0·118	104
Adh-1	−0·082	0·045	−0·033	104
Est-1	0·247	−0·007	0·242	104
Est-2	0·071	0·099	0·163	104
Mean	0·035	0·056**	0·089*	

* and ** indicate statistical difference from zero at probability levels of 0·05 and 0·01 respectively.

ces the number of generations of flies that can utilize it, is a function of temperature, of the size of the cladode, and most probably of its microflora. Rotting cladodes, both average and large in size, brought back to the laboratory and maintained at 25°C remained suitable as substrates for oviposition and larval feeding for three generations or more (unpublished observations). If adult flies tend to stay on suitable substrates rather than to disperse, and rots are 'colonized' by a small effective number of individuals, then the population-wide heterozygote deficiency measured by F_{IT} could be explained, at least in part, by inbreeding. The among-rot F_{ST} values suggest moderate levels of movement between rots within a patch of cactus. Experimental studies (Barker *et al.*, 1989) demonstrate the ability of *D. buzzatii* to move short distances (approximately 100 m) between cactus patches. In addition, data from several other cactus-utilizing species of *repleta* group

Table 10 *F*-statistics on adult flies emerging from six rots at Borallon, Queensland in November 1978. Only rots yielding at least ten individuals were included in the analysis

Locus	F_{IS}	F_{ST}	F_{IT}	*N*
Pgm	0·011	−0·014	−0·003	90
Aldox	1·000	0·000	1·000	90
Hex	−0·027	0·024	−0·002	90
Adh-1	0·173	0·043	0·208	90
Est-1	0·189	0·034	0·217	90
Est-2	0·176	0·067	0·232	89
Mean	0·153***	0·048***	0·193***	

*** indicates statistical difference from zero at a probability level of 0·001.

Table 11 *F*-statistics on adult flies emerging from 38 rots at Hemmant, Queensland in November 1978. Only rots yielding at least ten individuals were included in the analysis

Locus	F_{IS}	F_{ST}	F_{IT}	*N*
Pgm	0·005	0·038	0·042	810
Aldox	0·238	0·030	0·261	810
Hex	−0·046	0·026	−0·019	806
Adh-1	−0·157	0·034	−0·118	809
Est-1	0·204	0·060	0·252	809
Est-2	0·142	0·034	0·171	806
Mean	0·065	0·037***	0·100**	

** and *** indicate statistical difference from zero at probability levels of 0·01 and 0·001 respectively.

Table 12 Proportion of significant ($P < 0·05$) 2-locus linkage disequilibrium coefficients among flies emerging from 94 rots at Trinkey, O'Hara and four sites in Queensland

Locus	*Aldox*	*Est*-1	*Est*-2	*Hex*	*Adh*-1
Pgm	0	0·096	0·064	0·032	0·053
Aldox		0·138	0·043	0·043	0·064
Est-1			0·170	0·074	0·085
Est-2				0·074	0·064
Hex					0·106

Table 13 Variance components of linkage disequilibrium coefficients for Trinkey rots (June 1986)

	Within subpopulation components		Between subpopulation components		Total population component
Loci compared	D^2_{IS}	D'^2_{IS}	D^2_{ST}	D'^2_{ST}	D^2_{IT}
Pgm & Aldox	0·00036	0·07736	0·05705	0·00022	0·07758
Pgm & Hex	0·00241	0·03687	0·03428	0·00177	0·03864
Pgm & Adh-1	0·00249	0·04338	0·02438	0·00116	0·04454
Pgm & Est-1	0·00169	0·11160	0·05634	0·00000	0·11160
Pgm & Est-2	0·00023	0·15131	0·03560	0·00017	0·15148
Aldox & Hex	0·00075	0·06036	0·06016	0·00043	0·06079
Aldox & Adh-1	0·01907	0·06428	0·04470	0·00921	0·07349
Aldox & Est-1	0·00806	0·13793	0·07254	0·00196	0·13989
Aldox & Est-2	0·01244	0·17293	0·06033	0·00234	0·17527
Hex & Adh-1	0·00866	0·04564	0·03057	0·00200	0·04764
Hex & Est-1	0·00636	0·10366	0·05687	0·00000	0·10367
Hex & Est-2	0·02924	0·14304	0·04283	0·00914	0·15217
Adh-1 *& Est*-1	0·00829	0·10047	0·04331	0·00028	0·10075
Adh-1 *& Est*-2	0·00265	0·11102	0·03098	0·00000	0·11102
Est-1 *& Est*-2	0·01189	0·20591	0·05336	0·00892	0·21483

Drosophila suggest a strong tendency for the flies to remain on suitable substrates (Johnston and Heed, 1975; Johnston and Heed, 1976; Johnston and Templeton, 1982; Templeton and Johnston, 1982; Thomas, unpublished observations).

Analysis of the allozyme genotypes of flies emerging from rots collected from nature are consistent with a small number of "founders" on each substrate. Significant heterogeneity of allozyme frequencies is observed among rots collected in an area of less than 1 hectare, and there is generally a deficiency of heterozygotes relative to Hardy-Weinberg proportions (the exception is *Adh*-1, where there is a tendency towards an excess of heterozygotes).

Values of F_{ST}, a measure of coancestry, are significantly greater than zero in all collections of rots from all sites and are three to ten or more times the values for the seasonal collections, indicating substantial differentiation among rots within sites. Analysis of 2-locus disequilibria for rot emergences reveals significant "disequilibria" between the pairs *Aldox* and *Est*-1, *Est*-1 and *Est*-2, and *Hex* and *Adh*-1. In the seasonal collec-

Table 14 Variance components of linkage disequilibrium coefficients for O'Hara rots (June 1986)

	Within subpopulation components		Between subpopulation components		Total population component
Loci compared	D^2_{IS}	D'^2_{IS}	D^2_{ST}	D'^2_{ST}	D^2_{IT}
Pgm & Aldox	0·00052	0·01631	0·00899	0·00020	0·01650
Pgm & Hex	0·00095	0·01560	0·00663	0·00037	0·01597
Pgm & Adh-1	0·00050	0·01923	0·01095	0·00016	0·01939
Pgm & Est-1	0·00018	0·01699	0·01026	0·00006	0·01705
Pgm & Est-2	0·00074	0·02484	0·01087	0·00031	0·02515
Aldox & Hex	0·00210	0·01805	0·00929	0·00035	0·01840
Aldox & Adh-1	0·00205	0·01943	0·01027	0·00006	0·01949
Aldox & Est-1	0·00247	0·02051	0·01306	0·00002	0·02053
Aldox & Est-2	0·00114	0·02345	0·01067	0·00008	0·02361
Hex & Adh-1	0·00470	0·01979	0·00858	0·00049	0·02028
Hex & Est-1	0·00878	0·02198	0·00982	0·00080	0·02278
Hex & Est-2	0·00322	0·02331	0·01087	0·00182	0·02513
Adh-1 *& Est*-1	0·00507	0·02426	0·01067	0·00002	0·02428
Adh-1 *& Est*-2	0·00654	0·02985	0·01046	0·00144	0·03130
Est-1 *& Est*-2	0·00153	0·02029	0·01227	0·00024	0·02053

tions, however, these pairs do not show significant disequilibria. The members of each pair are located on the same chromosome, though *Hex* and *Adh*-1 show no linkage. This suggests that a small number of founders is responsible for the flies emerging from a rot.

Ohta (1982*a*) provides a model with which to subdivide the variance of linkage disequilibrium, in a manner analogous to the partitioning of *F*-statistics and of the inbreeding coefficient. She assumes Wright's (1969) island model of population structure, an assumption that, at the scale applied here, seems reasonable. By comparing various within and between subpopulation components of variance of disequilibrium, it is possible to distinguish drift due to limited dispersal, systematic epistatic selection and unequal systematic disequilibrium where selection for specific allele pairs occurs in only a few subpopulations. Results for pairs of loci from each collection of rots are consistent with drift due to limited dispersal. Total variance of disequilibrium within each collection of rots is 10 to 20 times that of the seasonal collections. Given that the number of individuals sampled over all the rots in a collection is large, these results are another indication of the small effective population sizes within rots.

What is the genetically effective number of individuals contributing to the progeny emerging from a rot? There are a numnber of factors that complicate attempts to answer this question rigorously. If a small number of males and females contribute to a rot, we expect the sexes to differ in their gene frequencies (Robertson, 1965) which would result in their progeny showing an excess of heterozygotes. This effect could easily be masked by the inbreeding that occurs at the level of rots. Possible selective differences resulting from the ageing of rots between generations may well be a factor also, as mentioned below. Crude estimates of N_e, using F_{ST}, under the assumptions of the island model with high migration rates, suggest that about ten individuals contribute gametes to reach rot.

Selection almost certainly influences the fate of alleles at these loci and closely linked regions. Ruiz *et al.* (1986) showed that viability selection on rearrangements of the second chromosome was operating in opposite directions at different stages of the life-cycle. *Est*-1, *Est*-2 and *Aldox* are located in or near the second chromosome arrangements, but Knibb and Barker (1988) have shown that apparent selection affecting allele frequencies at *Est*-2 cannot be explained by selection acting on the chromosome arrangements. It is likely, but remains to be demonstrated, that there are selective differences between rots. Thomas (unpublished) has shown that rots vary in the quality of nutrition that they provide for larvae in nature. The microflora differs from rot to rot (Barker *et al.*, 1984) and there is genetic variation in the flies' preferences for oviposition sites (Barker *et al.*, 1986; Barker unpublished). It is not known if there is genetic variation among larvae in their preferences for food, but they do discriminate between yeasts (Barker *et al.*, 1988).

Birley and Haley (1987) have recently investigated gametic disequilibria in populations of *D. melanogaster* of recent natural origin. Using caged populations with three food media, they simulated fine-scale spatial variation of the environment, and counted the allozyme genotypes in cages with various combinations of environments. Their results demonstrate epistatic selection in response to a novel environment, and show that this is not due to isolation between subpopulations. Rather the observed gametic disequilibria are produced by natural selection in the overall cage environment. Thus, even without any tendency to remain on suitable substrates, the observed spatial variation in the environment of *D. buzzatii* could well be exerting epistatic natural selection on the populations.

Hoffmann and Nielsen (1985) have investigated a model of polymorphisms in a situation like that found in *D. buzzatii.* To maintain a polymorphic balance, the model requires a positive correlation between fitness and the amount of genetic variation present in the individuals within a substrate. Inspiration for this model comes in part from experiments comparing the number of progency emerging from pure and mixed cultures (Beardmore, 1963; Kojima and Haung, 1972; Marinkovic and Ayala, 1975; Perez-Tomé and Toro, 1982). All these studies indicate an association between fitness and the amount of genetic variation present. A plausible possibility in the case of *D. buzzatii* is that the use of resources in a rot is more efficient, when more genetically variable larvae are present. The values of F_{ST} observed in the present study are consistent with about five pairs contributing to each rot. While this number is believable, it remains to be determined if the model is appropriate.

In conclusion, our data show that a small number of individuals contribute progency to a given rot. Other studies suggest that this sort of population structure is very common, especially among insects (*e.g.*, Hoffmann and Nielsen, 1985; Lacy, 1983). Measures of inbreeding strongly suggest

that flies mate within rots, thereby making mating non-random in the population as a whole. This is consistent with ecological observations on related species of *Drosophila*, showing that flies tend to remain on suitable substrates rather than dispersing to new substrates. Such conditions could make habitat selection a potent force in maintaining genetic variation in *D. buzzatii*. It remains to show directly the importance of habitat selection. Experiments to distinguish the effects on the maintenance of genetic variation of resource subdivision *per se* and spatial heterogeneity in the composition of those resources are needed.

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