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THE EVOLUTIONARY HISTORY OF *DROSOPHILA BUZZATII*. XIII. RANDOM DIFFERENTIATION AS A PARTIAL EXPLANATION OF CHROMOSOMAL VARIATION IN A STRUCTURED NATURAL POPULATION

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Natural populations of *Drosophila* species do not occupy a uniform environment but are faced with a finite number of discrete, ephemeral, and heterogeneous patches, each used as a breeding site by a small number of individuals (Heed 1968; Barker 1977; Jaenike and Selander 1979; Parsons 1982; Shorrocks 1982; Spieth 1982; Brncic 1983; Lachaise and Tsacas 1983; Lacy 1983; Hoffmann et al. 1984). This fact may profoundly affect the way natural selection works on genetic variation and, conversely, the results we obtain when trying to detect and measure selection. Taking advantage of the reasonably well known ecology of the cactophilic species *Drosophila buzzatii*, we have undertaken a long-term study to assess the relative importance of the different selective components operating on its inversion polymorphism in nature. Our first results (Ruiz et al. 1986) showed that differential larval viability seems to be the most important component acting on the second-chromosome polymorphism at the locality of Carboneras (Almería, southeastern Spain). We reached this conclusion by comparing the karyotype and/or inversion frequencies among samples from five different life-cycle phases. In this paper, we analyze the larval-stage data by means of *F* statistics to take into account the putative effect that the exploitation by *D. buzzatii* of discrete and ephemeral breeding sites may have on its chromosomal variation. In particular, we address the following questions. Is there significant heterogeneity for inversion frequencies among breeding sites? If so, has genetic differentiation taken place at random or, on the contrary, is it necessary to invoke selection for some inversions? Finally, how many mating pairs contribute on the average to each breeding site?

Drosophila buzzatii feeds and breeds on the decaying arms, cladodes, and fruits of several cactus species of the genus *Opuntia* (prickly pear; Carson and Wasserman 1965; Barker and Mulley 1976; Fontdevila et al. 1981, 1982). In the population studied at Carboneras (for a complete description, see Ruiz et al. 1986), *O. ficus-indica* is the only cactus species present. This platyopuntia is about 3–5 m tall and

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has a woody trunk and many spineless, oblong joints (cladodes) 20–40 cm wide and 30–60 cm long (Britton and Rose 1963; Bravo-Hollis 1978). When the study was carried out (late spring), the plants were in flower and *D. buzzatii* was breeding exclusively on the decaying cladodes. The cladodes have an estimated weight of about 0.5–2 kg, which sets an upper limit for the amount of substrate available at each breeding site in a given moment. We do not know exactly how much time a rotting cladode remains as a suitable breeding site in the field. Barker et al. (1983, 1986*a*) reported that in Australia, where *D. buzzatii* also occurs, most *Opuntia* cladode rots are quite ephemeral in late spring, drying out in 6–8 wk. Our experience, based on field observations, is that at Carboneras they are usable during one or, at most, two generations in late spring. This is corroborated by laboratory studies showing that *O. ficus-indica* is a relatively fast-rotting cactus: the pH of a fresh cladode inoculated with yeast and bacteria changes from 5 to 8 in 6 days at 25°C (F. Peris, unpubl. data).

Our analysis of the hierarchical structure of the *D. buzzatii* population rests on the following model. We assume that the adult population is large and that in each generation a limited number of females (n_f) lay eggs on individual rotting cladodes. After the progeny develop, all emerging adults mate at random, giving rise to the breeding population of the next generation. Following Kirby (1975), three life stages can be considered within a single breeding site: breeding adults; zygotes produced by random union of gametes; and third-instar larvae derived from the array of zygotes. The karyotypic frequencies in the third stage, which are those we analyze, are a function of (1) the number of breeding adults, which are a sample of the total adult population; (2) the frequencies in the zygotes, which can be a sample of all the zygotes produced by the n_f inseminated females; (3) the random mortality during the period from the egg to the third-instar larva; and (4) the differential larval viability among karyotypes. The above population model is based on two main assumptions: the population is large, and it is mating at random. Although at present no definitive data exist, the postulated large population size is supported by the observation that nearly 2000 adults were trapped within 2 h at the study locality (Ruiz et al. 1986). Moreover, estimates carried out in October 1978 using the method of capture-mark-release and recapture (Fontdevila et al., unpubl. data) indicated a population size of about 250,000 *D. buzzatii* adults, which is surely an overestimate of the population size throughout the year since, at that time (early fall), both *Opuntia* fruits and cladode rots were present. It is also worth noting that the inversion frequencies are rather stable over time at Carboneras (Ruiz 1982; Ruiz et al., unpubl. data). The assumption of random mating is sustained by the close agreement to the Hardy-Weinberg expectations for the karyotypic frequencies of both the second and fourth chromosomes in the progeny of wild-inseminated females (Ruiz et al. 1986).

MATERIAL AND METHODS

The collecting site is an old *Opuntia ficus-indica* plantation of approximately 1.5 ha that has been described in detail elsewhere (Ruiz et al. 1986). About 400 rotting cladodes were collected and brought to the laboratory from June 3 through 10, 1981. Forty rots found to host *Drosophila buzzatii* larvae were used for cytological analysis of salivary chromosomes. Each cladode was opened with a scalpel, and the rotting plant tissues were spread until we obtained a thin layer on which the larvae could be easily seen. Since all third-instar larvae were picked up, except for the few cases in which the karyotype could not be ascertained under the microscope, the sampling error at the time of survey was practically nil. For the present analysis, only the 27 rots with 10 or more third-instar larvae are considered. Polytene chromosomes were prepared by extracting the salivary glands of third-instar larvae in ethanol-acetic acid (3:1), squashing and staining them with lactic-acetic-orcein for 30 min. At Carboneras, *D. buzzatii* is polymorphic for four arrangements on the second chromosome (2st, 2j, $2jz^3$, and $2jq^7$) and two arrangements on the fourth (4st and 4s) (Fontdevila et al. 1981).

Data Analysis

The genetic structure of *D. buzzatii* may be ascertained by means of Wright's (1943, 1951) fixation indexes (F_{IS}, F_{IT}, and F_{ST}). These indexes were defined by Wright as the correlations between uniting gametes relative to the subpopulations (F_{IS}) and relative to the total population (F_{IT}). F_{ST} is the correlation of random gametes within subdivisions relative to the total population. As discussed by Long (1986) and Nei (1986), the application of the *F*-statistic model to real-world populations is controversial. Weir and Cockerham (1984) have given estimation procedures for the *F*-statistics parameters that do not make assumptions concerning the number of populations, the sample sizes, or the number of alleles observed per locus. However, their method assumes the absence of selection. In this study, we are dealing with inversions for which large differences in adaptive values exist in *D. buzzatii* (Ruiz et al. 1986) and many other *Drosophila* species (Dobzhansky 1970). Therefore, Weir and Cockerham's method does not seem appropriate here.

Nei (1977) showed that *F* statistics can be reformulated and defined as functions of observed and expected heterozygosities, rather than as correlations of uniting gametes. In Nei's theory, *F* statistics can be applied to any situation whether or not selection occurs and no matter how many alleles are segregating at a locus (chromosome). Nei's (1977) method (see also Nei and Chesser 1983) ignores population size differences because population size is quite transitory (which is especially true in this study), and we are generally interested in gene-frequency differences between populations, disregarding the effect of population size. However, one of the problems in applying the *F* statistics arises from unequal sample sizes in the subdivisions; since we are working with population frequencies, this difficulty can be overlooked. In this sense, the formulas given by Nei (1977), which are based on population allele frequencies, are directly applicable to our situation. The notation in this paper is the same as that used by Nei (1977), where the subscript *i* refers to the *i*th population (cladode rot) and the subscript *k* refers to the *k*th allele (gene arrangement) when there are more than two alleles (arrangements) at a locus (chromosome).

TABLE 1

ABSOLUTE KARYOTYPE FREQUENCIES AND ESTIMATES OF F_{ISi} FOR THE SECOND AND FOURTH CHROMOSOMES IN THIRD-INSTAR LARVAE OF *DROSOPHILA BUZZATII* TAKEN DIRECTLY FROM ROTTING CLADODES OF *OPUNTIA FICUS-INDICA*

ROT	SECOND CHROMOSOME												FOURTH CHROMOSOME				
No.	st/st	st/j	j/j	st/jz^3	j/jz^3	jz^3/jz^3	st/jq^7	j/jq^7	jz^3/jq^7	jq^7/jq^7	Total	F_{ISi}	st/st	st/s	s/s	Total	F_{ISi}
60	3	19	7	4	3		1		1		38	−0.2118	17	21		38	−0.3818
4	2	11	2	1			3				19	−0.3443	11	8		19	−0.2667
14	4	7	3	1				2			17	−0.0149	9	8		17	−0.3077
8	6	9	4	3			3	2			27	−0.0246	17	4	6	27	0.6447
103		5	2		3		1	1			12	−0.3408	8	3	1	12	0.2421
12	3	5	1	1			2	2			14	−0.1429	9	5		14	−0.2174
102	2	10	4	1			1			1	19	−0.0679	14	5		19	−0.1515
59		7	3	1	1						12	−0.3846	3	7	2	12	−0.1748
101	1	7	1		1						10	−0.4679	6	4		10	−0.2500
5	4	11	9	2	2					1	29	0.1040	23	6		29	−0.1154
32	7	5	1		1		1	1			16	0.0483	10	4	2	16	0.3333
106	4	3	4	2			2				15	0.2308	10	3	2	15	0.4410
105	5	12	2	1	2		4	5		1	32	−0.1344	21	9	2	32	0.1312
114	1	5	1	1			6	3		1	18	−0.2301	14	4		18	−0.1250
113	2	4	4	1			1	1			13	0.0945	8	4	1	13	0.1333
108	2	6		2			2		1		13	−0.3427	6	7		13	−0.3684
118	3	4	4	1	3		1	4			20	0.0133	9	10	1	20	−0.1905
111	3	7	1	2	1		5				19	−0.2527	12	5	2	19	0.2720
109	4	6	1					1			12	−0.1275	5	6	1	12	−0.1250
119	2	4	4		1						11	0.1200	7	4		11	−0.2222
200	5	14	11	5	7	2	5	5	3	1	58	0.0385	34	21	3	58	−0.0137
206	1	15	3		1		5	5			30	−0.3648	21	9		30	−0.1765
202	5	10	3		3		2	1			24	−0.0956	12	11	1	24	−0.1604
201	1	3	2	2	1		1	2			12	−0.0964	8	3	1	12	0.2421
204					1			3	1	5	10	−0.0870	10			10	—
56	1	7	1				1	1			11	−0.4143	7	4		11	−0.2222
19	2	4	6		1		1	2			16	0.1049	4	10	2	16	−0.2698

RESULTS

Within-Rot Analysis

Table 1 shows the raw data obtained from the cytological analysis of the third-instar larvae taken from the *Opuntia* rots. For each individual, the karyotype at both the second and the fourth chromosome was recorded. The unified fixation index for all gene arrangements in the *i*th rot (F_{ISi}) is also shown for the two chromosomes. This index is defined as the ratio of the difference between the expected and observed heterozygosities to the expected heterozygosity (Nei 1977).

For the second chromosome, 19 of 27 rotting cladodes (70%) show more heterokaryotypes than expected under Hardy-Weinberg. The same is true for the fourth chromosome in 18 of 26 rots (69%). Since the number of *Drosophila buzzatii* third-instar larvae present in a single rot in the Carboneras population is not very large (the average number is about 20), the power of statistical tests to prove the excess of heterokaryotypes within each rot would be weak (Brown 1970). However, these larvae might represent a reduced random sample of developing diploids drawn from a large zygotic population in Hardy-Weinberg proportions and produced by a high number (n_f) of inseminated females. If this were the case, the frequency of heterokaryotypes in the third-instar larvae would exceed the Hardy-Weinberg expectations on the average by an amount proportional to $1/(2N - 1)$, where N is the number of larvae analyzed (Levene 1949; Crow and Kimura 1970, pp. 55–56). If Levene's correction is introduced, a sign change in the weighted F_{ISi} value for the second chromosome takes place only in rot number 14 (from -0.0149 to 0.0149); all the other F_{ISi} values remain qualitatively identical. As pointed out by Majumder and Chakraborty (1981), if the estimated gene frequencies are moderate and if sample sizes are not too low, one might expect the observed proportions of heterozygotes to deviate above (or below) expectations about 50% of the time. A sign test indicates that the excess of heterokaryotypes when Levene's correction is applied is statistically significant for the fourth chromosome ($P < 0.05$) and marginally significant for the second chromosome ($0.10 > P > 0.05$). It seems reasonable to conclude that the process of sampling from a hypothetical zygotic population in Hardy-Weinberg proportions within each rotting cladode is not the (only) factor that generates the observed excess of heterokaryotypes. This conclusion is reinforced by the analysis of F statistics for the entire population (see below).

Another sampling process could explain the within-rot deviations of karyotypic frequencies from Hardy-Weinberg. If the number of inseminated females (n_f) breeding on a single rot is relatively small, the frequency of heterokaryotypes will exceed the Hardy-Weinberg expectations by a proportion $1/2N^*$, where N^* is the effective number of parents (Robertson 1965; see also Rasmussen 1979). The population structure of *D. buzzatii* suggests the existence of such founder effects associated with the colonization of *Opuntia* rots. Of course, selective differences among karyotypes in the viability component of the egg to the third-instar larva may also account for the negative values of F_{ISi}, but this point is discussed later.

TABLE 2

F STATISTICS FOR THE SECOND- AND FOURTH-CHROMOSOME ARRANGEMENTS OF *DROSOPHILA BUZZATII* AT CARBONERAS (ALMERÍA, SPAIN)

Arrangement	Average Frequency ($\bar{p}$)	P†	$\bar{p}^2$	$\overline{p^2}$‡	F_{ISk}	F_{ITk}	F_{STk}
Second chromosome							
st	0.4074	0.1434	0.1660	0.1810	−0.1661***	−0.0936	0.0621
j	0.4178	0.1565	0.1746	0.1861	−0.1278**	−0.0744	0.0473
jq^7	0.1134	0.0256	0.0129	0.0300	−0.0528	0.1264	0.1701
jz^3	0.0613	0.0013	0.0038	0.0054	−0.0733	−0.0435	0.0278
Weighted average					−0.1267	−0.0475	0.0703
Fourth chromosome							
st	0.7730	0.5982	0.5975	0.6060	−0.0467	0.0040	0.0484
s	0.2270	0.0522	0.0515	0.0600			

† Average frequency of homozygotes over the 27 rotting cladodes with an equal weight.
‡ Average of p^2 over the 27 rotting cladodes with an equal weight.
** $P < 0.01$.
*** $P < 0.001$.

Among-Rots Analysis

If the *O. ficus-indica* rots suitable for breeding are colonized by a limited number of inseminated females of *D. buzzatii*, a heterogeneous distribution of chromosome-arrangement frequencies would be expected to arise among rots. The population of third-instar larvae at Carboneras was analyzed by χ^2 contingency tables for heterogeneity of gene-arrangement frequencies among rots for both the second and fourth chromosomes. The computed χ^2 value for the second chromosome indicated a highly significant differentiation among rotting cladodes ($\chi^2 = 186.26$, df = 78, $P < 0.001$). Partition of the χ^2 showed that this differentiation has occurred mainly for the 2st and 2jq^7 gene arrangements ($\chi^2 = 49.20$, df = 26, $P < 0.01$; $\chi^2 = 114.53$, df = 26, $P < 0.001$). (For the fourth chromosome, $\chi^2 = 40.68$, df = 26, $P < 0.05$.)

A note of caution should be added to the preceding analysis. The computed χ^2 can be inflated by a factor approximately equal to N/N^*, where N is the number of larvae dissected from a rot and N^* the number of parents from which these larvae were descended, if the larvae from a single rot tend to be related (see Jaenike and Selander 1979). An estimation of the effective number of parents breeding on a single rot is provided in the last subsection of the "Results."

Analysis of Population Structure of 'D. buzzatii' by 'F' Statistics

The average chromosome-arrangement frequencies, as well as the average homokaryotypic frequencies, are given in table 2. From these data, the F_{IS}, F_{IT}, and F_{ST} values for each gene arrangement were computed. The same relative size in all rots was used for calculations (see the "Material and Methods" section). Since we are dealing with population frequencies and have no basis to presume Hardy-Weinberg equilibrium within each rot (surely the within-rot zygotic population is not in Hardy-Weinberg), we did not use Levene's correction in the computation of karyotype frequencies.

Table 2 shows that F statistics for the second chromosome vary with gene arrangement. All the $F_{\mathrm{IS}k}$ values are negative, indicating an excess of heterokaryotypes within rots. Nei (1965) pointed out that the F_{IS} parameter is not necessarily the same for all genotypes in a multi-allelic locus, except in the case of random differentiation. In this work, random differentiation is used to mean that genotype frequencies in a patchy environment are affected only by founder events associated with the colonization of each discrete site by a limited number of parents. Thus, if there is differential selection for genotypes, we could expect heterogeneity of F_{IS} values. Comparisons with Hardy-Weinberg expectations for each arrangement can be tested by using the formula $\chi^2 = N\,F^2_{\mathrm{IS}ik}$ (Li and Horvitz 1953), where N is the number of third-instar larvae analyzed. The χ^2 test for the deficiency of homokaryotypes shows that the $F_{\mathrm{IS}k}$ values for the gene arrangements 2st and 2j are significantly different from zero, whereas those for $2\mathrm{jq}^7$ and $2\mathrm{jz}^3$ are not. These results strongly suggest two likely evolutionary factors causing a significant deficiency of homokaryotypes for these chromosome arrangements: differential fitnesses among karyotypes counted after the operation of selection (probably the viability component of the egg to the third-instar larva), and a founder effect caused by only a few females' ovipositing on a single rot.

All but one of the $F_{\mathrm{IT}k}$ values in table 2 are negative and are accounted for by relatively low levels of heterogeneity of chromosome-arrangement frequencies among rots and/or relatively high levels of deficiency of homokaryotypes within rots. For the $2\mathrm{jq}^7$ arrangement alone, the heterogeneity among rots is high enough to overcompensate for the deficiency of homokaryotypes within rots, and the entire third-instar-larvae population shows an excess of the $2\mathrm{jq}^7/2\mathrm{jq}^7$ karyotype. The weighted averages of F_{IS}, F_{IT}, and F_{ST} for the second chromosome clearly show an overall excess of inversion heterokaryotypes in the whole population, which cannot be explained solely by the founder effect associated with the colonization of each rotting *Opuntia* cladode. This corroborates our previous analysis using Levene's method (in Dobzhansky and Levene 1948), which detected a significant excess of heterokaryotypes in the third-instar larvae (Ruiz et al. 1986). The heterogeneity of F_{ST} values for the second chromosome suggests that the differentiation of gene-arrangement frequencies has not occurred at random. Though we now have no statistical framework for testing hypotheses about F_{ST}, DeSalle et al. (1987) provided the following statistic to test the significance of F_{ST} values:

$$V = 4\,\Sigma_i\, n_i(a_i - \bar{a})^2,$$

where a_i is the arcsin-square-root transformation, measured in radians, of the frequency of a particular variant type at site i; n_i is the number of haploid genomes at site i; and $\bar{a}$ is the weighted average of a_is. The V statistic is distributed as a χ^2 with $r - 1$ degrees of freedom, where r is the total number of collecting sites. Using statistic V in our sample of 27 rots gives the following values: for arrangement 2st, $V = 73.80$, $P < 0.001$; for 2j, $V = 39.99$, $P < 0.05$; for $2\mathrm{jq}^7$, $V = 96.84$, $P < 0.001$; and for $2\mathrm{jz}^3$, $V = 41.48$, $P < 0.05$. As can be seen, all of the V statistics

TABLE 3

CORRELATIONS BETWEEN ARRANGEMENT FREQUENCIES OF THE SECOND CHROMOSOME OF *DROSOPHILA BUZZATII*

CHROMOSOME-ARRANGEMENT PAIR	CORRELATION	
	Expected	Observed
st-j	−0.7024	−0.2275
st-jq^7	−0.2965	−0.6061
st-jz^3	−0.2119	−0.4542
j-jq^7	−0.3030	−0.5894
j-jz^3	−0.2156	−0.0527
jq^7-jz^3	−0.0914	0.1554

yield significant χ^2 values, which are particularly high for arrangements 2st and 2jq^7. Thus, there is evidence for heterogeneity among rots.

The randomness of differentiation can be tested by a method based on the work of Nei (1965), comparing the observed and the expected correlations between gene frequencies. As Li (1969) stated, in subdivision with respect to multiple alleles, any particular covariance may be positive, negative, or zero, depending on the pattern of subdivision of the total population. However, if differentiation occurs at random, the covariances between two gene frequencies are always negative (Nei 1965). In this case, the expected correlation between the gene frequencies of the *m*th and *n*th alleles (gene arrangements) is given by the following expression (Nei 1965; see also Nei and Imaizumi 1966):

$$r_{(m,n)} = - [\bar{p}_m\bar{p}_n/(1 - \bar{p}_m)(1 - \bar{p}_n)]^{1/2} .$$

Table 3 shows the expected and the observed correlations; the observed correlations were obtained from the variances and covariances of gene-arrangement frequencies. The observed and expected correlations differ, although statistical tests of significance involving correlation coefficients (Sokal and Rohlf 1981, pp. 583–591) show that we can reject the null hypothesis only for the 2st-2j gene-arrangement pair ($t = 3.07$, $P < 0.01$). The lower-than-expected correlation for the pair 2st-2j can be related to the results of Ruiz et al. (1986), which showed that arrangement 2j significantly decreases in frequency from eggs to third-instar larvae, whereas arrangement 2st did not show a significant change. These results, coupled with the previously detected heterogeneity among F_{IS} values, suggest that part of the differentiation of inversion frequencies for the second chromosome of *D. buzzatii* in the population of third-instar larvae at Carboneras has not taken place at random.

The *F* statistics for the fourth chromosome are also shown in table 2. The F_{IS} value indicates an excess of 4st/4s heterokaryotype within rots, but the χ^2 test for Hardy-Weinberg deviations is not statistically significant. However, the heterogeneity of gene-arrangement frequencies among rots cancels out the heterokaryotype excess, making the F_{IT} value practically zero. The most likely conclusion is that founder events associated with the colonization of individual rots by a limited number of parents make up the most important factor for the genetic differentiation of the fourth chromosome in the population of third-instar larvae at Carboneras.

Number of Parents Breeding on a Single 'Opuntia ficus-indica' Rot

Differences in gene frequencies may arise by chance between sexes when a limited number of parents breed on a single rot. This causes an apparent within-rot excess of heterozygotes of the order of magnitude of half the square of the gene-frequency difference between the sexes. Robertson (1965) has shown that the expected frequency of heterozygotes in such cases is

$$H = 2pq(1 + 1/2N^*),$$

where p and q are the gene frequencies for a diallelic locus in the entire population (the result can also be extended to a multi-allelic locus) and N^* is the effective number of parents. In applying this formula, we must assume that no differences between sexes exist for gene-arrangement frequencies in the whole adult population of *D. buzzatii*. This assumption is surely true for the fourth-chromosome polymorphism when no statistical indications of fitness differences among karyotypes exist (the inversion frequencies are rather stable during the life cycle) and when the egg population from females taken in the wild fits the Hardy-Weinberg proportions almost perfectly (Ruiz et al. 1986).

The F_{IS} value for the fourth chromosome was estimated at -0.0467 (table 2). Taking this as the expected value, the number of parents breeding on a single rot can be obtained by taking $F_{\mathrm{IS}} = -1/2N^*$ (Long 1986, p. 639), which also follows from Nei's (1977) definition of fixation index (a more precise expression would be $F_{\mathrm{IS}} = -1/(2N^* - 1)$; Kirby 1975, pp. 35–37). Thus, the number of females that oviposit on a breeding site is approximately 5. It should be emphasized that since we are dealing with karyotypic frequencies in third-instar larvae, which are a sample of the zygotic population, the above estimate must be taken with caution.

The preceding analysis provides evidence that the number of third-instar larvae found in a rotting cladode (average number about 20) is probably greater than the corresponding number of their parents. As discussed in the subsection "Among-rots analysis," this means that the computed χ^2 for heterogeneity of inversion frequencies is inflated by a factor approximately equal to N/N^*, which can be closely taken as 1.95 (the total number of larvae analyzed in the 27 rots equals 527, and we are assuming that each rot is colonized by 5 singly inseminated females, which gives a total of 270 parents). Using this figure, the corrected values for the second chromosome are $\chi^2 = 95.52$, df = 78, $0.10 > P > 0.05$, and for the fourth chromosome, $\chi^2 = 20.86$, df = 26, $0.9 > P > 0.5$. The partitioned values for the 2st and $2jq^7$ gene arrangements, where df = 26, are $\chi^2 = 25.23$ ($0.9 > P > 0.5$) and $\chi^2 = 58.73$ ($P < 0.001$), respectively. The same reasoning also applies to the V statistic used above.

DISCUSSION

Several findings are relevant from the present analysis of the *Drosophila buzzatii* population structure at Carboneras: (1) a limited number of parents breed on each site; (2) in the third-instar-larva stage, a significant excess of heterokaryotypes for the second-chromosome-inversion polymorphism exists, and this excess is attributable to differential viability among karyotypes; and (3) part of the differentiation of gene-arrangement frequencies for the second chromosome in the third-instar-larvae population does not seem to have taken place at random. Concerning the first two points, an underlying assumption in the analysis is that differences in inversion frequencies between sexes do not exist in the breeding adults; thus, the zygotic population will be in Hardy-Weinberg proportions. This is surely true in the case of the fourth-chromosome polymorphism, in which rather stable gene-arrangement frequencies are observed throughout the life cycle and no statistically significant indication of selection was detected in any of the selective components investigated (Ruiz et al. 1986). However, some evidence of sex-dependent selection for late fitness components (virility or male mating success and fecundity) was detected for the second chromosome (Ruiz et al. 1986). Thus, an excess of heterokaryotypes in the whole zygote population is expected for this chromosome, which may contribute to the overall excess of heterokaryotypes in the third-instar-larva stage. Nevertheless, the excess arising from this source should be very weak since we did not detect a significant deficit of homokaryotypes in a large random egg sample obtained from females inseminated in the wild (Ruiz et al. 1986).

An additional assumption is made in our analysis: zygotes settle at random into each of the breeding sites; that is, no oviposition-behavior differences among karyotypes exist. Although differences in pH, temperature, volatiles, and yeast species among rotting *Opuntia* cladodes do exist (Barker 1982; Fogleman 1982; Starmer et al. 1982; Vacek 1982; Barker et al. 1983), the consequences of this heterogeneity at the karyotype level are uncertain. Barker et al. (1981*a*,*b*) tested the attractiveness of naturally occurring cactophilic yeast species for *D. buzzatii* adults in both laboratory and field experiments. They found that yeast species differentially attracted adult flies, but no significant oviposition preference was observed. For one of three polymorphic loci located on the second chromosome (*Esterase-2*), significant differences among alleles were detected for attractiveness although no consistent effects over experiments were seen. In these studies, attraction for feeding and/or oviposition are confounded, and Vacek et al. (1985) have tried to distinguish between them. From laboratory experiments in which flies were given a choice of only bacteria or bacteria plus one of eight yeast species, these authors concluded that adults have practically identical feeding and oviposition preferences for yeast species, and their results are consistent with the hypothesis that adults are polyphagous on yeasts but have yeast preference for oviposition. On the other hand, Vacek (1982) pointed out that flies feed randomly with respect to their genotype, but his data suggest that *D. buzzatii* females having different genotypes oviposit on yeast in a nonrandom manner. These results have been further supported by Barker et al. (1986*b*), who showed that adults of different genotypes for seven polymorphic enzyme loci do not have any preferences for feeding on particular yeasts, but females of different genotypes apparently prefer particular yeasts for oviposition. However, we are considering a rotting *Opuntia* cladode as a unit for oviposition, and some results clearly indicate heterogeneity for yeast species within a single rot (Peris, pers. comm.). Studies carried out by Starmer (1982) on agria cactus (*Stenocereus gummosus*) and by Fogleman and Starmer (1985) on organ-pipe cactus (*S. thurberi*) showed that a major source of variability in a yeast community is between different plants, but Starmer (1982) stated that variation between plants is most likely a result of samples representing different successional stages of the rotting process. Since cactophilic *Drosophila* females lay their eggs early in the rotting process (Starmer et al. 1986), reliable estimates of heterogeneity within and among rots at the moment a rot is optimal for oviposition are still lacking. Yeast diversity should be unequivocally apportioned into these two components if we want to analyze the role that habitat choice plays in nature. The ephemerality of *Opuntia* rots and the high dispersal rate of *D. buzzatii* adults (Fontdevila et al., unpubl. data) leads us to believe that it is difficult to establish a stable genetic polymorphism that depends on oviposition preferences. Nevertheless, this is an open question, and no definitive answer can be given at present.

An interesting result is that the observed correlation of frequencies of the 2st-2j inversion pair, together with the heterogeneity of F_{IS} values for gene arrangements on the second chromosome, do not match the expected correlations derived from models of random differentiation. The discrepancy between the observed and expected correlations has most probably arisen because selection in nature is acting strongly on the second chromosome; putative larval viability is the most important fitness component (Ruiz et al. 1986). Comparison of inversion frequencies between life-cycle stages has revealed significant changes from egg to third-instar larvae for arrangements 2j and $2jq^7$, with 2j decreasing and $2jq^7$ increasing during the larval stage. Arrangements 2st and $2jz^3$ showed the same pattern as 2j and $2jq^7$, respectively, but in these cases the differences were not statistically significant (Ruiz et al. 1986). An additional point is that viability values could be dissimilar among rotting *Opuntia* cladodes, and this may stress the differences in gene-arrangement frequencies of third-instar larvae. This raises the possibility that environmental heterogeneity is contributing to the maintenance of inversion polymorphism in *D. buzzatii* (Levene 1953; Hedrick et al. 1976; Taylor and Powell 1977). Niche specificity in this species might suggest that immature stages experience their environment as relatively fine-grained (Levins and MacArthur 1966; Levins 1968), but this is surely erroneous even for larvae inhabiting a single rot. Studies on *Opuntia* yeast communities in Australia (Barker et al. 1983) and Spain (Peris, unpubl. data) indicate heterogeneity in time (seasons of the year) and space (among rots); yet, variation between rots could be due to successional stages of the rotting process, as pointed out above (Starmer 1982). Interestingly, Fogleman et al. (1981, 1982) found that larvae of the cactophilic species *D. mojavensis* contain in their guts nonrandom samples of the yeasts available in their natural substrates. This result clearly shows that larvae are capable of distinguishing between patches of different yeast species. For *D. buzzatii*, Vacek (1982) has demonstrated a positive correlation of selective feeding on particular yeasts with developmental rate and viability of larvae.

Hoffmann and Nielsen (1985) analyzed the effect of population subdivision, generated by founding effects associated with the colonization of ephemeral breeding sites, on genetic variation. Their model relates to facilitation effects, which are an advantage in the raising of genetically variable progeny because genotype fitness is a function of the presence and relative frequencies of other coexisting genotypes (Clarke 1979; Pérez-Tomé and Toro 1982; Antonovics and Ellstrand 1984; Ellstrand and Antonovics 1985; Fowler and Partridge 1986). In their paper, Hoffmann and Nielsen (1985) investigated the establishment of an initially rare allele when selective differences among genotypes are determined by the genetic variance in each breeding site. The conclusion is that resource subdivision may be important in maintaining genetic variability when five or fewer mating pairs contribute to a breeding site. Our results point out that the effective number of parents breeding on each rot is within this critical range. If there is interference between individuals of like genotypes, a positive correlation between genetic variation and productivity could be expected from rots records. As a matter of fact, this correlation is evident from table 1 if we compare the number of larvae in a rot with the number of second-chromosome karyotypes present ($r = 0.80$, $P < 0.01$). However, differences in the number of females ovipositing on each rot would produce the same correlation, and more-direct experiments are required to test for frequency- and density-dependent interactions between competing larvae. Such experiments would provide insights into the nature of chromosomal variation in *D. buzzatii* in relation to the differential use of patches by the several karyotypes.

SUMMARY

Drosophila buzzatii is associated with several cactus species of the genus *Opuntia* (prickly pear). The rotting cladodes of *Opuntia* constitute discrete and ephemeral breeding sites colonized each generation by a finite number of mature *D. buzzatii* females. The genetic consequences of this population structure on the chromosomal variation in a natural population have been investigated by means of F statistics. Several conclusions are drawn from the present study: (1) the average number of parents breeding on a single *Opuntia* cladode can be estimated as 10; (2) there is a significant within-cladode excess of inversion heterokaryotypes for the second chromosome (since this excess is still significant when the whole population is considered, it cannot be explained solely as a result of the founder effect associated with the colonization of each cladode); and (3) F_{IS} and F_{ST} values for gene arrangements are heterogeneous on the second chromosome, which suggests that, for this chromosome, differentiation of inversion frequencies among breeding sites has not occurred at random. These results strongly point to the existence of differential fitnesses (attributable to the viability component of the egg to the third-instar larva) among karyotypes in nature, which agrees with previous analyses. Several assumptions underlying this analysis and the possible type of selection operating in the population are discussed.

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