Inversion and allozyme polymorphism show contrasting patterns of microgeographical population structure in a natural population of *Drosophila buzzatii* from Argentina

P. J. FERNÁNDEZ IRIARTE,*† C. RODRÍGUEZ† & E. HASSON†

**Laboratorio de Genética, Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Buenos Aires, Argentina*

†GIBE, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Buenos Aires, Argentina

Keywords:

allozymes;
cactus hosts;
Drosophila;
genetic structure;
inversion polymorphism;
natural selection.

Abstract

Second chromosome inversions and genotypic frequencies at seven allozyme loci were determined in a natural population of the cactophilic species *Drosophila buzzatii* that uses as breeding sites the necrotic cladodes of the prickly pear *Opuntia quimilo* and the rotting stems of cardón, *Trichocereus terschekii*. Different processes govern the evolutionary fate of inversion and allozyme polymorphisms. A pattern of heterotic balance for inversions seems to be acting uniformly in each breeding site and could depend on different regimes of density-dependent selection within cactus hosts. Patterns of variation of allozymes revealed significant heterogeneity in allele frequencies for Esterase-1 (*Est-1*) among *O. quimilo* rots and Aldehyde oxidase (*Aldox*) and Xanthine dehydrogenase (*Xdh*) among *T. terschekii* substrates and showed gene-cactus effects only for Esterase-2 (*Est-2*). Consistent and significant excesses of homozygotes were detected at both the within-rot and in the total population levels that could be accounted for by diversifying selection among individual breeding sites.

Introduction

One of the major goals of evolutionary genetics is to explain the maintenance of genetic variation. Variable selection in different patches of a heterogeneous environment may decrease the chances of the loss of a polymorphism, and thus, may be a plausible mechanism contributing to the maintenance of genetic variation (Barker, 1990; Hedrick, 1990; Powell, 1992, 1997). Conditions that have been suggested for the maintenance of genetic variation by environmental heterogeneity are: (1) genotype–environment interaction (Levene, 1953; Hedrick, 1986) (2) soft-selection (De Meeus *et al*., 1993; Santos, 1997) and (3) habitat choice (García-Dorado, 1986; Powell, 1992) or habitat selection (Barker, 1990). However, theoretical studies have shown that when selection coefficients are small the maintenance of variation is extremely restricted (Hoekstra *et al*., 1985).

Though at first sight it seems a simple idea, some complications arise because the way in which a population is adapted to environmental heterogeneity depends upon the scales of the environment, the relative frequency of environmental patches and the fitness of the various genotypes in each patch (Levins, 1968). In fact, habitats may be heterogeneous in many dimensions and experimental approaches for studies of multiple niche selection can be greatly facilitated by the identification of the actual dimensions to which genetic variation responds.

If our goal is to examine the relevance of heterogeneous environments, not all species of *Drosophila* are equally appropriate because in most cases we are unaware of the relevant ecological dimensions affecting population structure (Hey & Houle, 1987). Cactophilic species such as *D. buzzatii* (buzzatii complex-repleta group, Ruiz & Wasserman, 1993) have emerged as excellent model systems for such ecological–genetic studies (Fontdevila, 1989, 1995; Barker, 1990). This South American species, which probably originated in the Argentinian Chaco (Carson & Wasserman, 1965;

Correspondence: Pedro J. Fernández Iriarte, Laboratorio de Genética, Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3250, (7600) Mar del Plata, Buenos Aires, Argentina.
Fax: 54223-475-3150; e-mail: firiarte@mdp.edu.ar

Fontdevila, 1989), has successfully colonized the Mediterranean area (Fontdevila, 1989) and Australia (Barker, 1982) in historically recent times. *Drosophila buzzatii* breeds and feeds upon the necrotic tissues of several endemic *Opuntia* species, such as *O. quimilo, O. sulphurea, O. vulgaris* and columnar cacti such as *Trichocereus terschekii*, *T. pasacana* and *Cereus validus* in Argentina (Hasson *et al*., 1992). Thus the possibility of exploiting different types of cactus hosts provides the basis of habitat selection as a result of variable selection regimes among different host plants (Barker, 1990; Fernández Iriarte & Hasson, 2000; Rodríguez *et al*., 2000). Moreover, potential cactus hosts may differ in several biologically relevant variables for the flies such as chemical composition (Kircher, 1982), diversity of the microflora associated with the rotting process (Starmer *et al*., 1990) and rot size, larval density and rot duration (Hasson *et al*., 1992).

Previous work in *D. buzzatii* has shown not only that polymorphic second chromosome inversion arrangements affect several life history traits such as viability and developmental time but also provided suggestive evidence of the dependence on the cactus host in which larvae were grown (Fernández Iriarte & Hasson, 2000 and references therein). For instance we have shown that the ancestral arrangement 2*ST* and the derived 2*J* had opposite effects on both viability and developmental time, and that these effects were not independent of the cactus host.

Surveys of electrophoretic variation in *D. buzzatii* populations have shown for certain loci, some of them in linkage disequilibrium with the inversion polymorphism, significant microspatial (among rots within localities) genetic heterogenity (Barker *et al*., 1986a; Sokal *et al*., 1987, 1998; Quezada-Díaz *et al*., 1997). In particular, allelic variation at *Est-2*, which is tightly linked to the inversion system, was shown to covary with several rot related variables such as rot-age, pH and number of flies emerged from each rot (Barker *et al*., 1986a). Moreover, field experiments provided evidence that *Est-2* genotypes are differentially attracted to yeast species, providing the first indication of possible genotype specific choice of yeasts (Barker, 1981, 1990).

In this paper, we report a study of comparative microspatial population genetic structure, using second chromosome inversions and seven linked allozyme loci as genetic markers, in a natural population in which *D. buzzatii* breeds upon two types of resources, the prickly pear *O. quimilo* and the cardón *T. terschekii*. Our aim is to investigate the possible effect of habitat heterogeneity on the spatial distribution of variation at both inversion and allozyme polymorphisms. Specifically we address the following questions: (a) Are flies carrying different inversion karyotypes or electrophoretic genotypes differentially attracted to both cactus hosts?; (b) Are fitness differences among karyotypes or electrophoretic genotypes constant across cactus hosts?; (c) Are patterns of population structure for the inversion polymorphism and linked electrophoretic variation independent?; (d) Are the patterns of distribution of genetic variation among rotting cladodes within cactus host analogous in both host plants?

Materials and methods

The present study was performed in the locality of Chumbicha (28.8°S, 66.3°W) in an area 60 km south of San Fernando del Valle de Catamarca city in the eastern slopes of Sierras de Ambato (Catamarca Province, Argentina). *Opuntia quimilo* (OQ) and *Trichocereus terschekii* (TT) are the most abundant cactus species in the site of collection, although other potential hosts, such as *O. sulphurea* and *Cereus validus,* are also present. However, during the time of the experiments rotting cladodes of OQ and decaying stems of TT were the only breeding resources available.

Sampling procedures

We investigated several life cycle stages by collecting two different kinds of samples: (1) natural substrates (rots) of OQ and TT and (2) adult flies (resident adults).

Natural substrates: two different life cycle stages were sampled from naturally ocurring rots:
(a) Third instar larvae: larvae were directly collected from the fermenting tissues of 10 OQ (sample size ranged from 10 to 38 individuals) and 7 TT (10–54 individuals) rots and, (b) Newly emerged adults (eclosed adults): emerging adults were recovered from two independent sets of rots. All flies emerging from each rot were aspirated daily during 15 days after rot collection, sexed and kept in vials until further processing. Random pairs of adults emerged from each one of the first set of six OQ and 1 TT rots (33 for OQ and 26 for TT) were placed in individual vials. The analysis of 11 progeny larvae from each cross allowed to infer the karyotype of both parents. Flies emerged from the second set of 10 OQ (sample size ranged from 31 to 38 flies) and 8 TT (10–115 flies) were electrophoretically assayed for seven allozyme loci (see below).

Resident adults: two kinds of baits, prepared with rotting materials of OQ or TT, were used as attractants for adult flies and were distributed at random. Flies were collected using an insect aspirator and those sampled in each kind of bait were processed separately. Females were placed individually in vials containing 5–6 mL of killed yeast *Drosophila* medium. The analysis of one progeny larva from each vial allowed the estimation of inversion frequencies in the progeny of wild females. Two additional sets of flies collected in each kind of bait were electrophoresed in order to estimate genotype and allele frequencies in samples of resident adults.

Cytological and electrophoretic methods

Salivary gland chromosomes were prepared according to standard procedures and inversion karyotypes identified using the cytological maps described for *D. buzzatii* (Ruiz & Wasserman, 1993). Adults were assayed for: Esterase 1 (*Est-1*), Esterase 2 (*Est-2*), Aldehyde Oxidase (*Aldox*), Xanthine Dehydrogenase (*Xdh*), Leucyl Amino Peptidase (*Lap*), Peptidase-1 (*Pep-1*) and Peptidase-2 (*Pep-2*). Electrophoretic techniques and allele nomenclature follow Barker & Mulley (1976), Barker *et al*. (1986a) and Rodríguez *et al*. (2000).

All loci map to the polymorphic second chromosome of *D. buzzatii*. *Aldox* and *Est-1* are located inside the segments rearranged by inversions 2*J* and 2*JZ3*; *Est-2* is outside the segment rearranged by 2*J* but very close to the proximal break point and inside 2*JZ3*. *Aldox* maps between *Est-1* and *Est-2* in the 2ST arrangement, whereas in arrangement $2JZ^3$ the order of these loci is *Est-2–Est-1–Aldox*. *Pep-2*, *Lap* and *Xdh* are outside the segments rearranged by inversions (Schafer *et al*., 1993; Betrán *et al*., 1995; Ranz *et al*., 1997).

Statistical methods

Data were first arranged in multiway tables and analysed by means of log-linear models (Bishop *et al*., 1975) in order to explore and infer the ecological and genetic factors affecting the microgeographical distribution of genetic variation (Santos *et al*., 1999). In the analysis of multiway tables we test a hierarchical set of models starting with the most complex (Sokal & Rohlf, 1985). The resulting log-likelihood statistics (G) were used as the basis of a χ^2 analysis.

For the analysis of the inversion polymorphism the raw data consisted of a three way contingency table in which individuals were classified according to (i) the individual rot and (j) the cactus species (*O. quimilo* vs. *T. terschekii*) from which each individual was sampled and (k) arrangement. The log-linear model in this case is

$$f_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk}$$

where f_{ijk} is the expected frequency in cell ijk ($i = 1,\ldots, n$ rots; $j = 1$, 2 cactus hosts and $k = 1$–3 karyotypes), μ is the grand mean of the logarithms of the probabilities and α, β and γ are the main effects of the variables defined by the subscript in the formula, and the remaining terms are, in order, the first order interactions and the three way interaction. Although in the population studied three arrangements are segregating at polymorphic frequencies namely 2ST, 2*J* and $2JZ^3$, the latter two were pooled into a single class referred to as 2*J** due to the low numbers of $2JZ^3$ carriers.

Analyses of allozyme variation were done on a two-allele collapse of the data in order to avoid empty cells. The most common alleles at multiallelic loci were retained and all the others pooled. An array of 10 four-way contingency tables was employed, with rots ($i =$ 1–n), cactus ($j = 2$), locus 1 ($k =$ 1–2 alleles) and locus 2 ($l =$ 1–2 alleles) as main sources of variation *Lap* and *Pep-1* were excluded from these analyses because the most common alleles of these loci had frequencies very close to 0.9 The log-linear model in these analyses is:

$$f_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \alpha\beta_{ij} + \alpha\gamma_{ik} + \alpha\delta_{il} + \beta\gamma_{jk} + \alpha\beta_{ij} + \beta\delta_{jl} + \gamma\delta_{kl} + \alpha\beta\gamma_{ijk} + \alpha\gamma\delta_{ikl} + \beta\gamma\delta_{jkl} + \alpha\beta\gamma\delta_{ijkl},$$

where the new terms, as compared with the previous model, correspond to the additional first and second order interactions, and the third and fourth order interactions.

In log-linear models, we are interested primarily in testing for the presence of interactions rather than the main effects. For instance, the term $\beta\gamma_{jk}$ (or $\beta\delta_{jl}$) can be defined as representing the interaction between cactus hosts and locus 1 (or locus 2). In these cases, if we assume that genotypic frequencies in the zygotes entering the rots of both cactus hosts do not differ significantly, a significant interaction points to differences in allele frequencies between cactus hosts, which might be interpreted as evidence of variable selection at locus 1 (or locus 2). Significant second order interaction term $\alpha\beta\gamma_{ijk}$ (or $\alpha\beta\delta_{ijl}$) may, in turn, indicate that the distribution of locus 1 (or locus 2) allele frequencies among rots is not independent of the cactus host.

An additional advantage of the log-linear analysis is that the term $\gamma\delta_{kl}$ which defines the interaction locus 1 × locus 2 over all rots and cactus hosts can be considered as a test of gametic disequilibrium (Weir, 1996).

Significance levels were adjusted by sequential Bonferroni tests for multiple comparisons according to Rice (1989).

Population genetic structure was analysed by means of *F*-statistics in order to investigate the distribution of variation within and among individual breeding sites (rots) for chromosomal and allozyme variation in samples of the larval and eclosed adults stages, respectively. Unbiased estimates of Hardy–Weinberg exact *P*-values were calculated by the Markow chain method. *F*-statistics 95% confidence intervals were obtained by means of a permutation tests over alleles. Population genetic software packages GENEPOP (Raymond & Rousset, 1995) and FSTAT (Goudet, 1995) were used for the analysis of population structure and LINKDOS (Black & Krafsur, 1985) for the estimation of two-locus linkage disequilibrium coefficients. The statistical software STATISTICA (Statsoft, Inc., 1996) was used for log-linear analyses.

Results

Patterns of variation of inversion frequencies between and within cactus hosts

Inversion frequencies were estimated in three different life cycle stages from samples of resident adults attracted to, larvae feeding in and adults emerged from the two

Table 1 Relative frequencies of second chromosome arrangements in samples of resident adults, third instar larvae and eclosed adults of *D. buzzatii* collected in association with rots of *Opuntia quimilo* and *Trichocereus terschekii*.

Arrangement	Resident adults	Third instar larvae	Eclosed adults
O. quimilo			
2ST	0.527	0.513	0.555
2*J*	0.408	0.377	0.320
2*JZ*^{3}	0.065	0.110	0.125
N	184	520	128
T. terschekii			
2ST	0.565	0.523	0.587
2*J*	0.340	0.326	0.269
2*JZ*^{3}	0.095	0.152	0.144
N	200	310	104

N: number of chromosomes sampled.

Table 2 Analysis of population genetic structure by means of *F*-statistics for the third instar larvae samples of *D. buzzatii* collected in 10 rots of *Opuntia quimilo* and seven rots of *Trichocereus terschekii*.

Arrangement	$F_{IS.k}$	$F_{IT.k}$	$F_{ST.k}$
O. quimilo			
2ST	–0.218	–0.188	0.025
2*J*	–0.229	–0.209	0.017
2*JZ*^{3}	0.035	0.053	0.019
All	–0.182**	–0.158**	0.021**
T. terschekii			
2ST	–0.147	–0.165	–0.016
2*J*	–0.159	–0.119	0.035
2*JZ*^{3}	–0.060	–0.015	0.042
All	–0.133*	–0.115	0.016

*$P < 0.05$, **$P < 0.01$.

main cactus hosts present in the natural population of Chumbicha (Table 1).

The results of the log-linear model designed to analyse patterns of variation of inversion frequencies across cactus hosts and rots showed a nonsignificant karyotype × cactus interaction ($G_2 = 1.32$, $P = 0.517$). This suggests that fitness differences among karyotypes are similar in OQ and TT, if we assume that attraction of flies and oviposition behaviour are independent of second chromosome karyotype. The absence of significant differences in inversion frequencies between samples of resident adults (Table 1) collected in traps prepared with rotting materials of OQ or TT ($\chi^2_2 = 2.48$, $P = 0.289$) gives support to the first assumption. The interaction karyotype × rot in the log-linear analysis, which was not significant ($G_{12} = 19.0$, $P = 0.088$) points out that patterns of differentiation of karyotypic frequencies among rots within host cacti were homogeneous.

The *F*-statistics analysis yielded negative and mostly significant $F_{IS.k}$ coefficients for 2ST and 2*J* in OQ (ST: $\chi^2_1 = 12.36$, $P < 0.01$; *J*: $\chi^2_1 = 13.63$, $P < 0.001$) and TT (ST: $\chi^2_1 = 3.35$, $P = 0.067$; *J*: $\chi^2_1 = 3.92$, $P < 0.05$) pointing to an overall excess of *ST/J* heterokaryotypes within rots in both cactus hosts (Table 2). Moreover, permutation tests showed that F_{IS}s averaged across arrangements were also significantly lower than 0 in both hosts (Table 2). F_{IT} values were also negative in OQ and TT but only significant in the former (Table 2). These results point out that the excess of heterokaryotypes at the level of individual rots does not cancel out when we consider the population as a whole. Differentiation among rots, as measured by F_{ST}, was significant in OQ but not in TT (Table 2).

We used the test statistic $\Delta p/[\text{Var}\ (\Delta p)]^{1/2}$ (where Δp is the difference between the frequency of a given arrangement in samples of two consecutive life cycle stages) devised by Anderson *et al*. (1979) to compare inversion frequencies (pooled across cactus species) between samples of third instar larvae and eclosed adults (Table 1). The results showed that the frequency of 2*J* decreased significantly ($\Delta p = -0.064$, $P = 0.033$) from the larval to the eclosed adult stage, whereas 2ST exhibited an opposite although not significant trend ($\Delta p = 0.052$, $P = 0.08$).

Patterns of allozyme variation between and within cactus hosts

Allele frequencies for all loci in samples of resident and eclosed adults associated to both cactus are given in Table 3.

The results of the log-linear analysis are summarized in Table 4. In general the best model varied according to the pair of loci analysed in each case. Second order interactions rot × cactus × locus 1 (or locus 2) was significant (wide matrix *P*-value: 0.05) for *Est-1* and *Xdh* in all contingency tests and for *Aldox* in two, indicating that the patterns of distribution of allele frequencies over rots are not independent of the cactus (Table 4). In addition, *Est-2* was invariably involved in significant cactus × locus interactions, suggesting that, despite genetic differentiation among rots within cactus (see below), OQ and TT are perceived as different patches of an heterogeneous environment by *Est-2* genotypes.

Either habitat choice, i.e. flies with different *Est-2* genotypes do not oviposit at random, or multiple niche selection (see discussion for details) are two possible explanations for this observation. Evidence giving support to habitat selection comes from comparisons of allele frequencies between samples of resident adults attracted to baits prepared with rotting materials of OQ or TT (Table 3) that were not significant for any of the loci assayed except *Est-2* ($\chi^2_3 = 13.93$, $P < 0.01$).

Finally, the locus 1 × locus 2 interaction, which provides a test for independence between loci, was significant in the comparisons involving *Est-1*–*Est-2* and

Table 3 Relative allele frequencies for seven allozyme loci in samples of resident and eclosed adults of *D. buzzatii* emerged from *Opuntia quimilo* and *Trichocereus terschekii* rots.

	Opuntia quimilo		*Trichocereus terschekii*	
Locus	Resident adults	Eclosed adults	Resident adults	Eclosed adults
Est-1				
N	172	578	132	388
a+	0.006	0.010	0.000	0.018
a	0.180	0.215	0.129	0.173
b	0.703	0.694	0.742	0.670
c	0.110	0.080	0.129	0.131
d	0.000	0.002	0.000	0.008
Est-2				
N	170	566	132	378
a+	0.000	0.004	0.000	0.011
a	0.147	0.161	0.159	0.201
b	0.724	0.537	0.582	0.434
c	0.124	0.240	0.182	0.257
d	0.006	0.058	0.076	0.098
Lap				
N	170	580	146	394
a	0.024	0.105	0.034	0.096
b	0.941	0.847	0.884	0.873
c	0.035	0.047	0.082	0.030
d	0.000	0.002	0.000	0.000
Aldox				
N	162	592	138	368
a+	0.043	0.012	0.014	0.065
a	0.741	0.699	0.790	0.726
b	0.210	0.274	0.188	0.198
c	0.006	0.014	0.007	0.011
Xdh				
N	122	558	94	372
a+	0.000	0.000	0.000	0.005
a	0.008	0.039	0.021	0.027
b	0.795	0.701	0.809	0.718
c	0.197	0.247	0.170	0.215
d	0.000	0.013	0.000	0.035
Pep-1				
N	170	582	142	394
a	0.035	0.034	0.035	0.023
b	0.900	0.885	0.901	0.931
c	0.065	0.077	0.063	0.046
d	0.000	0.003	0.000	0.000
Pep-2				
N	172	580	138	378
a+	0.000	0.045	0.000	0.032
a	0.360	0.391	0.391	0.418
b	0.622	0.522	0.580	0.534
c	0.017	0.041	0.029	0.016

N: number of alleles sampled.

Aldox–Xdh (Table 4), suggesting that these pairs of loci are in linkage disequilibrium. Further analysis of two-locus linkage disequilibrium coefficients (*D*) in individual rots showed that only 26 of 331 comparisons were significant, and that an important proportion of significant comparisons involved the same pair of loci (data not shown) detected in log-linear analysis.

The analysis of population genetic structure for electrophoretic variation showed an overall excess of homozygotes, i.e. positive F_{IS}s, at the level of individual rots, in six of seven loci: *Est-1*, *Est-2*, *Aldox*, *Xdh*, *Pep-1* and *Pep-2* in OQ, and only for *Est-2* and *Xdh* in TT (Table 5). Mean F_{IS}s averaged over loci were also positive and significant in both cactus hosts. However, F_{IS} values, considered as correlation coefficients (Sokal & Rohlf, 1985), were significantly heterogeneous among loci in OQ ($\chi^2_6 = 13.36$, $P < 0.05$), but not in TT ($\chi^2_6 = 8.74$, $P =$ n.s.).

In OQ, all loci showing significant excess of homozygotes at the level of individual rots also exhibited significant departures from Hardy–Weinberg expectations at the level of the total population, whereas in TT positive and significant F_{IT}s were observed for *Est-2, Lap and Xdh* (Table 5). Mean F_{IT}s averaged over loci were also positive and significant in both cactus hosts (Table 5).

Finally, among-rot differentiation was significant for *Est-2* and *Lap* in both OQ and TT, for *Est-1* only in the former and for *Aldox* and *Xdh* in the latter. Mean F_{ST} averaged over loci was also significant in both cactus species (Table 5).

Discussion

The way in which a population is adapted to environmental heterogeneity depends on different processes such as habitat selection, density-dependent selection and diversifying selection, that may lead to the maintenance of genetic variation. We showed that the maintenance of the inversion polymorphism and allozyme variation in the cactophilic *D. buzzatii* depends on different features of the cactus hosts that flies use as breeding sites in nature.

In *Drosophila* host selection includes two main components: settling and oviposition behaviour (Jaenike, 1990). Our study shows that flies carrying different second chromosome arrangements were not differentially attracted to *O. quimilo* and *T. terschekii*. Moreover, during the larval stage no evidence of differential performance in environmental patches was detected, suggesting that viability in the host plants is independent of the karyotype.

Our analysis of population structure revealed an excess of inversion heterokaryotypes in the larval stage. This excess may be because of the limited number of individuals colonizing each breeding site (Santos *et al.*, 1989; Thomas & Barker, 1990), however, the local excess at the level of individual rots should be cancelled out when the total population is considered. Thus, the overall excess of heterokaryotypes detected at the level of the total population can be considered as evidence against genetic

Table 4 Log-linear analysis: measures of partial association (G) (see text for explanation of factors involved in the analysis).

Locus 1–locus 2	Cactus × locus 1	Cactus × locus 2	Locus 1 × locus 2	Rot × cactus × locus 1	Rot × cactus × locus 2
Est-1–Est-2	0.47	5.43*	11.88*	16.23*	3.72
Est-1–Aldox	1.74	1.96	0.12	16.03*	15.48*
Est-2–Aldox	16.17*	2.07	1.02	6.09	13.15
Aldox–Xdh	3.10	0.95	40.86*	6.79	12.12*
Est-1–Xdh	2.11	0.08	0.88	15.56*	23.77*
Est-1–Pep-2	2.05	3.27	0.08	14.36*	5.70
Est-2–Xdh	10.17*	0.62	1.18	6.26	23.41*
Est-2–Pep-2	9.91*	3.10	0.01	5.70	5.14
Aldox–Pep-2	3.17	4.21	0.03	15.41*	6.43
Xdh–Pep-2	0.69	3.70	0.05	22.98*	5.42

*Significant at a wide matrix P-value: 0.05.

Table 5 Analysis of population genetic structure by means of F-statistics for seven allozyme loci in samples of eclosed adults of *D. buzzatii* emerged from 10 rots of *O. quimilo* (OQ) and eight rots of *T. terschekii* (TT).

	F_{IS}		F_{IT}		F_{ST}	
Locus	OQ	TT	OQ	TT	OQ	TT
Est-1	0.078*	0.023	0.098**	0.034	0.022**	0.012
Est-2	0.072*	0.133**	0.099**	0.161**	0.029**	0.032**
Lap	0.003	0.088	0.021	0.123*	0.018**	0.039*
Aldox	0.271**	–0.016	0.271**	0.015	0.001	0.030*
Xdh	0.158**	0.169**	0.158**	0.238**	0.001	0.083**
Pep-1	0.143**	–0.069	0.151**	–0.044	0.010	0.023
Pep-2	0.082*	–0.018	0.083*	–0.004	0.001	0.013
Average	0.115**	0.057**	0.126**	0.088**	0.012**	0.033**
Confidence interval	(–0.035 0.033)	(–0.041 0.046)	(–0.034 0.034)	(–0.043 0.043)	(–0.006 0.008)	(–0.009 0.011)

*$P < 0.05$, **$P < 0.01$.

drift, suggesting that heterokaryotype advantage may be a plausible explanation. Therefore, our results not only confirm the adaptive role of the inversion polymorphism in *D. buzzatii*, but also demonstrate that selection is playing a significant role in shaping the distribution of inversion frequencies within and between individual breeding sites of each generation.

Comparisons between samples of consecutive life cycle stages revealed the action of directional selection in favour of 2ST at the expense of 2*J* for pupal viability, in coincidence with previous observations in another natural population (Hasson *et al*., 1991). However, if populations are at equilibrium, pupal viability effects should be counteracted by selection of opposing sign acting through another fitness component (Ruiz *et al*., 1986). Interestingly, it has been shown that arrangement 2ST increases viability and decreases developmental time and body size, whereas the effects of 2*J* on these life history traits have opposite signs (Betrán *et al*., 1998; Fernández Iriarte & Hasson, 2000), suggesting that the advantage of the heterokaryotype *ST/J* may be the resultant of opposing selective forces in different life cycle stages.

The superiority of inversion heterokaryotypes has been recurrently invoked in several species of *Drosophila* (reviewed in Krimbas & Powell, 1992; Powell, 1992; Powell, 1997). All in all, our results suggest that soft selection may favour heterokaryotypes with superior competitive ability in nature where competition is certainly more intense (Fernández Iriarte *et al*., 1999), but not when karyotypes are raised in uncrowded conditions (Fernández Iriarte & Hasson, 2000). Furthermore, the host plants present in the population studied differ in various features, cardón rots are relatively less abundant and less ephemeral and provide larger breeding sites than *O. quimilo*, suggesting that conditions in *T. terscheckii* may be less stringent for the flies. Thus, competition is expected to be more intense in *Opuntia* rots than in cardón and consequently may favour genotypes with superior competitive ability such as heterokaryotypes. A comparable syndrome has been reported in *D. melanogaster* for *In(2L)t* which showed only evidence of overdominance when reared at high density and high temperature (Van Delden & Kamping, 1991). However, we should be cautious when interpreting this pattern, because the mere excess of heterozygotes does not necessarily imply heterosis (see Lewontin, 1974, p. 242).

The patterns of allozyme variation revealed a different picture. Our study suggest that *Est-2*, or linked loci, might be involved in differential attraction to cactus hosts and

that viability of *Est-2* genotypes may depend upon the type of resource, in agreement with the suggestion that this locus may be involved in habitat selection (Barker *et al*., 1986a,b; Sokal *et al*., 1987, 1998). All the information available points to an adaptive role of *Est-2* variation that may be associated with its function as an enzyme with detoxifying functions in the larval substrate (East, 1982). Moreover, the pattern of variation of *Est-2*, which appears as largely independent of the inversion system to which it is tightly linked in *D. buzzatii* (see also Rodríguez *et al*., 2000), resembles the situation of αGpdh in *D. melanogaster* which is located inside the segment rearranged by *In(2L)t*. This locus shows geographical clines that cannot be accounted for by hitchhiking with the inversion, but seems to be responding to environmental selection, in contrast to the *Adh* latitudinal clines that can be easily explained by its association with *In(2L)t* (Kamping & Van Delden, 1999). Still, in the case of *Est-2,* more powerful techniques, such as sequential gel electrophoresis, revealed an important amount of hidden variation (Barker, 1994), suggesting that we must await for nucleotide sequence data in order to improve our understanding of the selective processes shaping allelic variation at this locus.

The significant excess of homozygotes detected in our analysis of population structure for some of the allozyme loci in Chumbicha, either at the level of individual rots and in the total population, are in congruence with observations in Australian (Barker *et al*., 1986a; Thomas & Barker, 1990) and some Spanish (Betrán *et al*., 1995) populations of *D. buzzatii.* Presence of null alleles, inbreeding, Wahlund effect and diversifying selection are recurrent explanations for these observations (Barker & Mulley, 1976; Santos, 1994). First, two lines of evidence allow us to discard null alleles (i) extensive surveys of allozyme variation in Argentina showed that null alleles are virtually absent (Rodríguez *et al*. 2001) and (ii) at least for certain loci, namely *Aldox*, *Pep-1* and *Pep-2*, which showed a deficit of heterozygotes in OQ but not in TT. Secondly, the heterogeneity among F_{IS} for the different loci argues against genetic drift and inbreeding, because these two processes are expected to affect all polymorphic loci uniformly. However, since some of the loci assayed (*Aldox, Est-1* and *Est-2*) are tightly linked to the inversion polymorphism and other segregate nearly independent (e.g. *Xdh*), hitchhiking may be a plausible explanation for heterogeneity among fixation indices. Yet, recent surveys of electrophoretic variation in populations of *D. buzzatii* from Argentina, showed that in Chumbicha only *Est-1* and to a lesser extent *Aldox* are in linkage disequilibrium with the inversion system (Rodríguez *et al*., 2001).

Evidence against inbreeding or Wahlund effect includes (i) random mating with respect to rot of origin (Quezada-Díaz *et al*., 1992) (ii) that *Opuntia* breeding sites are suitable for only one generation before drying out (Santos *et al*., 1989; Quezada-Díaz *et al*., 1997) and (iii) low levels of linkage disequilibrium (present paper). Thus, our results gives support to Santos (1994) suggestion that diversifying selection is a reasonable explanation for the deficiency of heterozygotes.

Therefore, the relevant environmental variables most likely involved in the adaptive response of allozyme variation in the cactophilic *D. buzzatii* lie at the level of individual rots (Barker, 1990). Furthermore, analyses of microgeographical population structure have suggested that spatial heterogeneity may be one of the main factors promoting diversifying selection (Santos *et al*., 1989; Santos, 1994) probably because of differences in the diversity and composition of the microflora associated with the rotting process of cactus tissues (Barker *et al*., 1986a; Sokal *et al*., 1998).

In conclusion, the patterns of distribution of genetic variation at the microgeographical level in the *D. buzzatii* population of Chumbicha suggest that different processes govern the evolutionary fate of inversion and allozyme polymorphisms. Heterotic balance seems to be acting uniformly on the inversion polymorphism, leading to moderate differentiation among breeding sites, whereas some allozyme loci seem to be under some form of diversifying selection, with patterns of variation largely independent of chromosomal arrangements.

Acknowledgments

We thank A. Fontdevila and M. Santos for helpful discussions and critical reading of the manuscript, J. S. F. Barker for the critical reading of earlier versions of this paper and J. J. Fanara for assistance and insightful comments. The advice and suggestions of W. Anderson and one anonymous reviewer helped to improve the manuscript. We would also like to thank F. Latorre and C. Perez for assistance in the field. The last version of this manuscript was written while EH was on sabbatical leave at Departamento de Genética y Microbiología, Universidad Autónoma de Barcelona, with the support of Dirección General de Universidades (Ministerio de Educación, Cultura y Deporte, Spain). This work was supported by Universidad de Buenos Aires, CONICET and Universidad Nacional de Mar del Plata grants awarded to EH. PFI is fellow and EH is member of Carrera del Investigador Científico of CONICET (Argentina).

References

Anderson, W.W., Levine, L., Olvera, O., Powell, J.R., de la Rosa, M.E., Salceda, V.M., Gaso, M.I. & Guzmán, J. 1979. Evidence for selection by male mating success in natural populations of *Drosophila pseudoobscura*. *Proc. Nat. Acad. Sci. (USA)* **76**: 1519–1523.

Barker, J.S.F. 1981. Selection at allozyme loci in cactophilic *Drosophila*. In: *Genetic Studies of Drosophila Populations* (J. B. Gibson & J. G. Oakeshott, eds), pp. 161–184. *Proceedings of the*

1979 Kiola Conference, Australian National University, Camberra.

Barker, J.S.F. 1982. Population genetics of *Opuntia* breeding *Drosophila* in Australia. In: *Ecological Genetics and Evolution. The Cactus–Yeast–Drosophila Model System* (J. S. F. Barker & W. T. Starmer, eds), pp. 209–224. Academic Press, Sydney.

Barker, J.S.F. 1990. Experimental analysis of habitat selection and maintenance of genetic variation. In: *Ecological and Evolutionary Genetics of Drosophila* (J. S. F. Barker, W. T. Starmer & R. J. MacIntyre, eds), pp. 161–175. Plenum Press, New York.

Barker, J.S.F. 1994. Sequential gel electrophoretic analysis of esterase-2 in two populations of *Drosophila buzzatii*. *Genetica* **92**: 165–175.

Barker, J.S.F., East, P.D. & Weir, B.S. 1986a. Temporal and microgeographic variation in allozyme frequencies in a natural population of *Drosophila buzzatii*. *Genetics* **112**: 577–611.

Barker, J.S.F. & Mulley, J.C. 1976. Isozyme variation in natural populations of *Drosophila buzzatii*. *Evolution* **30**: 213–233.

Barker, J.S.F., Vacek, D.C., East, P.D. & Starmer, W.T. 1986b. Allozyme genotypes of *Drosophila buzzatii*: feeding and oviposition preferences for microbial species and habitat selection. *Aust. J. Biol. Sci.* **39**: 47–58.

Betrán, E., Quezada-Díaz, J.E., Ruiz, A., Santos, M. & Fontdevila, A. 1995. Linkage disequilibrium between allozymes and chromosome inversion in two colonizing populations. *Heredity* **74**: 188–199.

Betrán, E., Santos, M. & Ruiz, A. 1998. Antagonistic pleiotropy effect of second chromosome inversions on body size and early life-history traits in *Drosophila buzzatii*. *Evolution* **52**: 144–154.

Bishop, Y.M.M., Fienberg, S.E. & Holland, P.W. 1975. *Discrete Multivariate Analysis: Theory and Practice*. MIT Press, Cambridge, MA.

Black, W.C. & Krafsur, E.S. 1985. A Fortran program for the calculation and analysis of two-locus linkage disequilibrium coefficients. *Theor. Appl. Genet.* **70**: 491–496.

Carson, H.L. & Wasserman, M. 1965. A widespread chromosomal polymorphism in a widespread species *Drosophila*. *Am. Nat* **99**: 111–115.

De Meeus, T., Michalakis, Y., Renaud, F. & Olivieri, I. 1993. Polymorphism in heterogeneous environments, evolution of habitat selection and sympatric speciation: soft and hard selection models. *Evol. Ecol.* **7**: 175–198.

East, P.D. 1982. Non-specific Esterases of *Drosophila buzzatii*. In: *Ecological Genetics and Evolution. The Cactus–Yeast–Drosophila System* (J. S. F. Barker & W. T. Starmer, eds), pp. 323–336. Academic Press, Sydney.

Fernández Iriarte, P. & Hasson, E. 2000. The role of the use of different host plants in the maintenance of the inversion polymorphism in the cactophilic *Drosophila buzzatii*. *Evolution* **54**: 1295–1302.

Fernández Iriarte, P., Levy, E., Devincenzi, D., Fanara, J.J., Rodríguez, C. & Hasson, E. 1999. Temporal and spatial variation of the inversion polymorphism in two natural population of *Drosophila buzzatii*. *Hereditas* **131**: 93–99.

Fontdevila, A. 1989. Founder effects in colonizing populations. The case of *Drosophila buzzatii*. In: *Evolutionary Biology of Transient Unstable Populations* (A. Fontdevila, ed.), pp. 74–95. Springer-Verlag, Heidelberg.

Fontdevila, A. 1995. Genetics and ecology of natural populations. In: *Genetics of Natural Populations. The Continuing Importance of Theodosius Dobzhansky Evolutionary Biology of Transient Unstable Populations* (L. Levine, ed.), pp. 198–221. Columbia University Press, New York.

García-Dorado, A. 1986. The effect of niche preference on polymorphism protection in a heterogeneous environment. *Evolution* **40**: 936–945.

Goudet, J. 1995. FSTAT (Version 1.2): a computer program to calculate *F*-statistics. *J. Hered.* **86**: 485–486.

Hasson, E., Naveira, H. & Fontdevila, A. 1992. The evolutionary history of *Drosophila buzzatii* XXX. The breeding sites of the Argentinian species of the *Drosophila mulleri* complex (subgenus Drosophila- repleta Group). *Rev. Chilena Hist. Nat.* **65**: 319–326.

Hasson, E., Vilardi, J.C., Naveira, H., Fanara, J.J., Rodríguez, C., Reig, O.A. & Fontdevila, A. 1991. The evolutionary history of *Drosophila buzzatii* XVI. Fitness component analysis in an original natural population from Argentina. *J. Evol. Biol.* **4**: 209–225.

Hedrick, P.W. 1986. Genetic polymorphism in heterogeneous environments: a decade later. *Annu. Rev. Ecol. Syst.* **17**: 535–566.

Hedrick, P.W. 1990. Theoretical analysis of habitat selection and the maintenance of genetic variation. In: *Ecological and Evolutionary Genetics of Drosophila* (J. S. F. Barker, W. T. Starmer & R. J. MacIntyre, eds), pp. 209–227. Plenum Press, New York.

Hey, J. & Houle, D. 1987. Habitat choice in the *Drosophila affinis* subgroup. *Heredity* **58**: 463–471.

Hoekstra, R.F., Bijlsma, R. & Dolman, J. 1985. Polymorphism from environmental heterogeneity: models are only robust if the heterozygote is close in fitness to the favoured homozygote in each environment. *Genet. Res.* **45**: 299–314.

Jaenike, J. 1990. Factors maintaining genetic variation for host preference in *Drosophila*. In: *Ecological and Evolutionary Genetics of Drosophila* (J. S. F. Barker, W. T. Starmer & R. J. McIntyre, eds), pp. 195–207. Plenum Press, New York.

Kamping, A. & Van Delden, W. 1999. A long-term study on interaction between *Adh and* αGpdh allozyme polymorphisms and the chromosomal inversion *In (2L) t* in a seminatural population of *D. melanogaster*. *J. Evol. Biol.* **12**: 809–821.

Kircher, H.W. 1982. Chemical composition and its relationship to Sonoran desert *Drosophila*. In: *Ecological Genetics and Evolution. The Cactus–Yeast–Drosophila Model System* (J. S. F. Barker & W. T. Starmer, eds), pp. 143–158. Academic Press, Sydney.

Krimbas, C.B. & Powell. 1992. Introduction. In: *Drosophila Inversion Polymorphism* (C. B. Krimbas & J. R. Powell, eds), pp. 1–53. CRC Press, Boca Ratón, FL.

Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. *Am. Nat.* **87**: 331–333.

Levins, R. 1968. *Evolution in Changing Environments*. Princeton University Press, Princeton, NJ.

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

Powell, J.R. 1992. Inversion polymorhism in *Drosophila pseudoobscura* and *Drosophila persimilis*. In: *Drosophila Inversion Polymorphism* (C. B. Krimbas & J. R. Powell, eds), pp. 73–126. CRC Press, Boca Ratón, FL.

Powell, J.R. 1997. *Progress and Prospects in Evolutionary Biology. The Drosophila Model*. Oxford University Press, Inc., Oxford.

Quezada-Díaz, J.E., Laayouni, H., Leibowitz, A., Santos, M. & Fontdevila, A. 1997. Breeding structure of *Drosophila buzzatii* in relation to competition in prickly pears (*Opuntia ficus-indica*). *Genet. Sel. Evol.* **29**: 367–382.

Quezada-Díaz, J.E., Santos, M., Ruiz, A. & Fontdevila, A. 1992. The evolutionary history of *Drosophila buzzatii* XXV. Random mating in nature. *Heredity* **63**: 373–379.

Ranz, J.M., Segarra, C. & Ruiz, A. 1997. Chromosomal homology and molecular organization of Muller's elements D and E in the *Drosophila repleta* species group. *Genetics* **145**: 281–295.

Raymond, M. & Rousset, F. 1995. GENEPOP (*Version 1.2*). A population genetics software for exact tests and ecumenicism. *J. Hered.* **86**: 248–249.

Rice, W.R. 1989. Analizing tables of statistical tests. *Evolution* **43**: 223–225.

Rodríguez, C., Piccinali, R., Levy, E. & Hasson, E. 2000. Contrasting population genetic structures using allozymes and the inversion polymorphism in *Drosophila buzzatii*. *J. Evol. Biol.* **13**: 976–984.

Rodríguez, C., Piccinali, R., Levy, E. & Hasson, E. 2001. Gametic associations between inversion and allozyme polymorphisms in *Drosophila buzzatii*. *J. Hered.* **92**: 382–391.

Ruiz, A., Fontdevila, A., Santos, M., Seoane, M. & Torroja, E. 1986. The evolutionary history of *Drosophila buzzatii* VIII evidence for endocyclic selection acting on the inversion polymorphism in a natural population. *Evolution* **40**: 740–755.

Ruiz, A. & Wasserman, M. 1993. Evolutionary cytogenetics of the *Drosophila buzzatii* species complex. *Heredity* **70**: 582–596.

Santos, M. 1994. Heterozygote deficiencies under Levene's population subdivision structure. *Evolution* **48**: 912–920.

Santos, M. 1997. Resource subdivision and the advantage of genotypic diversity in *Drosophila*. *Heredity* **78**: 302–310.

Santos, M., Eisses, K.T. & Fontdevila, A. 1999. Competition and genotype-by-environment interaction in natural breeding substrates of *Drosophila*. *Evolution* **53**: 175–186.

Santos, M., Ruiz, A. & Fontdevila, A. 1989. The evolutionary history of *Drosophila buzzatii* XIII. Random differentiation as a partial explanation of chromosomal variation in a structured natural population. *Am. Nat.* **133**: 183–197.

Schafer, D.J., Fredline, D.K., Knibb, W.R., Green, M.M. & Barker, J.S.F. 1993. Genetics and linkage mapping of *Drosophila buzzatii*. *J. Hered.* **84**: 188–194.

Sokal, R.R., Oden, N.L. & Barker, J.S.F. 1987. Spatial structure in *Drosophila buzzatii* populations: simple and directional spatial autocorrelation analysis. *Am. Nat.* **129**: 122–142.

Sokal, R.R., Oden, N.L. & Thomson, B.A. 1998. Local spatial autocorrelation in biological variables. *Biol. J. Linn. Soc.* **65**: 41–62.

Sokal, R.R. & Rohlf, F.J. 1985. *Biometry*, 2nd edn. Freeman, San Francisco.

Starmer, W.T., Lachance, M., Phaff, H.J. & Heed, W.B. 1990. The biogeography of yeast associated with decaying cactus tissue in North America, the Caribean, and Northern Venezuela. In: *Evolutionary Biology* (M. K. Hecht, B. Wallace & R. MacIntyre, eds), Vol. 24, pp. 115–190. Plenum Press, New York.

Thomas, R.H. & Barker, J.S.F. 1990. Breeding structure of natural populations of *Drosophila buzzatii*: effects of the distribution of larval substrates. *Heredity* **64**: 355–365.

Van Delden, W. & Kamping, A. 1991. Changes in relative fitness with temperature among second chromosome arrangements in *Drosophila melanogaster*. *Genetics* **127**: 507–514.

Weir, B.S. 1996. *Genetic Data Analysis II*. Sinauer Assoc., Sunderland, MA.

Received 13 July 2001; revised 28 September 2001; accepted 14 December 2001