

Contrasting population genetic structures using allozymes and the inversion polymorphism in *Drosophila buzzatii*

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Abstract

Second chromosome inversion and genotypic frequencies at seven allozyme loci, differentially associated with inversions, were determined in seven natural populations of *Drosophila buzzatii*. The patterns of variation of allozymes and the inversion polymorphisms were significantly different, indicating the role of adaptive differentiation for the latter. Moreover, the patterns of population structure varied among allozyme loci, suggesting the operation of diversifying selection for certain loci. Differentiation was negligible for Leucyl-amino peptidase (*Lap*) and Peptidase-2 (*Pep-2*), low to moderate for Aldehyde oxidase (*Aldox*), Peptidase-1 (*Pep-1*) and Esterase-1 (*Est-1*) and high for Esterase-2 (*Est-2*) and Xanthine dehydrogenase (*Xdh*). Significant linkage disequilibria were detected between inversions and *Aldox*, *Est-1*, *Est-2* and *Xdh*. Multiple regression analyses of inversion and allele frequencies on environmental variables revealed the existence of clines for inversions, *Est-1*, *Est-2*, *Xdh* and *Aldox* along altitudinal, latitudinal and/or climatic gradients. Tests using conditional allele frequencies showed that *Est-1* and *Aldox* clines could be accounted for by hitchhiking with inversions, whereas natural selection should be invoked to explain the clines observed for *Est-2* and *Xdh*.

Introduction

The cactophilic *Drosophila buzzatii* (*buzzatii* complex – repleta group, Ruiz & Wasserman, 1993) is native to South America and has attained a subcosmopolitan distribution by following passively its native host plants of the genus *Opuntia*. Studies in natural populations have shown that second chromosome polymorphic arrangements affect life history traits and morphometric traits associated with fitness providing evidence of the adaptive role of the inversion polymorphism (Ruiz *et al.*, 1986; Hasson *et al.*, 1991, 1992; Ruiz *et al.*, 1991; Barbadilla *et al.*, 1994; Norry *et al.*, 1995; Fanara *et al.*, 1996, 1997; Norry *et al.*, 1997a,b; Rodríguez *et al.*, 1999; Fernández

Iriarte & Hasson, 2000). Clinal variation over large geographical areas can be taken as indirect evidence of strong selective forces (Endler, 1977). Previous work on *D. buzzatii* has shown that inversion frequencies are correlated with latitudinal gradients in different continents (Knibb & Barker, 1988; Hasson *et al.*, 1995). Moreover, in South America population structure is coincident with phytogeographical regions, suggesting that karyotype fitnesses vary in different areas (Ruiz, 1982; Hasson *et al.*, 1995).

Studies of allozyme variation in colonized populations from Australia and Spain have suggested a significant role of natural selection shaping allele frequency distributions for several loci, particularly Esterase-1 (*Est-1*), Esterase-2 (*Est-2*) and Aldehyde-oxidase (*Aldox*) (Barker & East, 1980; Barker *et al.*, 1986; Quezada-Díaz, 1993). However, these loci are tightly linked to the chromosomal segments rearranged by inversions (Quezada-Díaz, 1993; Schafer *et al.*, 1993; Betrán *et al.*, 1995). Thus, fitness differences among arrangements may be causally

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related to adaptive variation at loci or gene regions tightly linked to inversions. Conversely, fitness differences among *Est-1*, *Est-2* and *Aldox* genotypes could be the result of hitchhiking due to their association with an adaptive polymorphism. The analysis of variation of conditional allele frequencies allows the estimation of the amount of total variation that can be accounted for by geographical variation in inversions frequencies (Voelker *et al.*, 1978). Knibb & Barker (1988) reported that clinal variation of *Est-2* in Australian populations was also significant even when they considered conditional frequencies, suggesting that spatial variation for this locus cannot be accounted for by linked inversions.

In this paper, we report an analysis of population structure in *D. buzzatii* using second chromosome inversion polymorphism and seven polymorphic allozyme loci linked to inversions. One of our aims is to revisit the adaptive role of chromosomal variation by means of a comparative approach of population genetic structures and to assess the significance of direct selection and hitchhiking on electrophoretic variation.

Materials and methods

Seven populations located in three different phytogeographical regions belonging to the Argentinian Chaco Dominium (Cabrera, 1976) were sampled for this study (Table 1). The geographical location of the populations studied is shown in Fig. 1 and the geographical coordinates, altitude and diversity of potential host plants (i.e. *cactus* species) are given in Table 1.

Wild-adult flies were collected by net sweeping on fermenting banana baits. A grand total of more than 700 wild males were processed following the crossing scheme shown in Fig. 2. Briefly, wild males were crossed individually with virgin females of a laboratory stock carrying a system of balanced dominant mutations *Antennapedia* and *Delta 5* (*Antp*/ Δ^5 , see Schafer *et al.*, 1993 and Barker, 1994 for a description of the mutants) and homozygous for the most frequent alleles of the electrophoretic loci assayed (see below). After 48 h males were removed from the vials and preserved at -70°C . Vials with progeny were maintained until the emergence of adults. A single *Antp*-adult male of each F_1 progeny (*Antp*/+,



Fig. 1 Geographical location of the *D. buzzatii* natural populations sampled in the present study.

heterozygous for a wild chromosome) of each successful cross was individually backcrossed with *Antp*/ Δ^5 virgin females, and 48 h later recovered from the vials and preserved at -70°C . Each wild male was electrophoretically assayed simultaneously with the F_1 sons in order to check for the presence of null alleles.

In order to identify the chromosomal arrangement transmitted by each wild male to their corresponding F_1 sons we analysed the salivary gland chromosomes of five third instar larvae of the progeny of the backcrosses (F_2 hereafter). The cytological analysis of five F_2 individuals allows the identification of the wild chromosome with a high probability, because in the progeny two of the three genotypes are carriers of the wild chromosome and can

Table 1 Populations sampled in the present study along with their geographical coordinates (in $^\circ$), altitude (in m), the phytogeographical regions to which they belong and the *cactus* species, potential hosts, present.

Population	Region	Latitude	Longitude	Altitude	<i>Cactus</i> species
Chumbicha	Western Chaco	28.8	66.3	421	A, B, C, D, E
La Cebila	Western Chaco	28.8	66.3	850	A, E, D
Río Hondo	Western Chaco	27.5	65.9	290	A, B, C, D, F
Berna	Eastern Chaco	29.3	59.9	48	B, H
Pto. Tirol	Eastern Chaco	27.4	59.1	53	F, B, H
A. Escobar	Pampa	34.4	58.7	5	G
Otamendi	Pampa	34.3	58.8	10	G

A: *Trichocereus terchekii*; B: *Stetsonia corynne*; C: *Cereus validus*; D: *Opuntia quimilo*; E: *O. sulphurea*; F: *O. ficus indica*; G: *O. vulgaris*; H: *O. pampeana*.

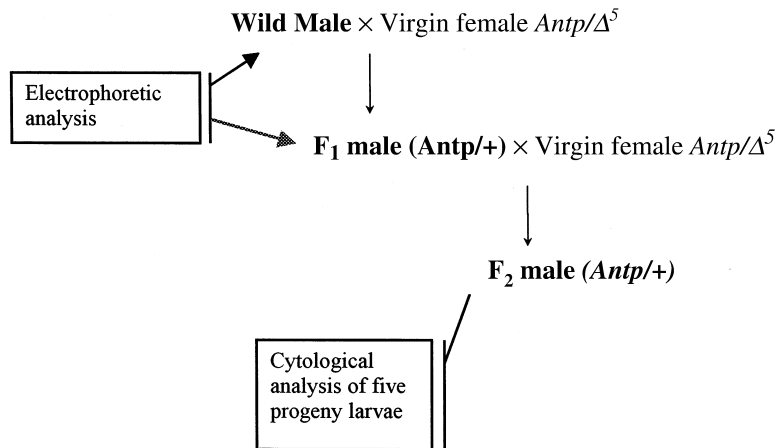


Fig. 2 Crossing scheme employed to isolate wild chromosomes from seven natural populations of *D. buzzatii*.

be readily identified due to the rearrangements present in the balancer chromosomes (J.S.F. Barker, personal communication).

All flies were electrophoretically assayed for seven allozyme loci: *Est-1*, *Est-2*, *Aldox*, Xanthine dehydrogenase (*Xdh*), Peptidase-1 (*Pep-1*), Peptidase-2 (*Pep-2*) and Leucyl-amino peptidase (*Lap*), according to the techniques and methodologies described in Barker & Mulley (1976), Barker *et al.* (1986), and Quezada-Díaz (1993).

Est-1 and *Aldox* are located inside the segments rearranged by inversions *J* and *Z*³, *Est-2* is outside the segment rearranged by inversion *J* but very close to the proximal break point and inside inversion *Z*³. *Aldox* maps between *Est-1* and *Est-2* in the Standard arrangement (2ST), while in arrangement 2JZ³ the order of these loci is *Est-2*–*Est-1*–*Aldox*. *Pep-2* (Betrán *et al.*, 1995), *Xdh* (Ranz *et al.*, 1997) and *Lap* (Schafer *et al.*, 1993) are outside the rearranged chromosomal segments. *Pep-1* is located in the second chromosome, but its exact location with respect to the inversion system is unknown.

Salivary-gland chromosomes were analysed according to the methodology described in Fontdevila *et al.* (1981).

Two hierarchical levels were considered in the analysis of population structure: populations within regions and regions within the total. Thus, total variation among demes (F_{DT}) can be partitioned in two components, variation among demes within regions (F_{DR}) and among regions within the total (F_{RT}) such that

$$(1 - F_{DT}) = (1 - F_{DR})(1 - F_{RT})$$

As chromosomal inversions are genetic factors restricting effective crossing-over in heterokaryotypes, chromosome arrangements can be considered as semi-isolated populations with restricted gene flow (recombination). Thus, arrangements within populations were considered at the basic level in an independent analysis of population structure designed to estimate the extent of genetic differentiation among arrangements within populations. In other words, arrangements were considered as sub-populations within populations. As the experimental

protocol allows us to know the genotypes of the electrophoretic loci and one of the inversion arrangements carried by each wild male, we considered each individual fly carrying a certain arrangement as the basic data for this level of the hierarchy. Thus, we define F_{CD} as a fixation index that measures the degree of genetic differentiation among arrangements.

Allele frequencies and *F*-statistics were estimated using the programme Genetic Data Analysis (GDA) (Weir, 1996). Bootstrap-simulation techniques were employed to test for the significance of *F*-statistics using the programme Fstboot (Van Dongen, 1995), which not only allows to test the statistical significance of each coefficient, but also to compare between *F*-statistics estimated for different loci.

We also investigated the degree of linkage disequilibrium between allozyme loci and inversions by means of contingency tests.

Multiple-regression analyses of genetic variables (inversion and allozyme frequencies) on geographical and climatic variables were employed to explore possible environmental factors of population genetic structure. The geographical variables included were latitude, longitude and altitude (data obtained from Instituto Geográfico Militar). Climatic data, averaged over the last decade, were mean annual temperature, humidity, atmospheric pressure and rainfall (obtained from Servicio Meteorológico Argentino).

Multiple regressions were performed using the programme Statistica (Stat soft 1995). Prior to the analysis allele frequencies were angularly transformed (Sokal & Rohlf, 1985).

Results

The raw data, i.e. allele and inversion frequencies in the populations sampled, are available upon request. All electrophoretic loci were polymorphic at the 0.01 criterion and the frequencies of null alleles were lower than 0.03.

Table 2 *F*-statistics for the hierarchical analysis of population structure (see text for explanation of the meaning of subindices).

	F_{CD}	F_{DR}	F_{RT}	F_{DT}
<i>Lap</i>	+0.008	+0.006	+0.000	+0.005 (0.265)
<i>Pep-2</i>	+0.006	-0.004	+0.009	+0.002 (0.374)
<i>Est-1</i>	+0.043	+0.025	-0.004	+0.022 (0.001)
<i>Pep-1</i>	+0.013	+0.018	-0.013	+0.010 (0.050)
<i>Aldox</i>	-0.048	+0.014	+0.014	+0.040 (0.000)
<i>Est-2</i>	+0.073	+0.004	+0.077	+0.069 (0.000)
<i>Xdh</i>	+0.075	-0.014	+0.072	+0.064 (0.000)
Enzyme (average)	+0.008	+0.009	+0.039	+0.038 (0.001)
Inversion polymorphism		+0.042	+0.230	+0.247 (0.000)

Statistical probability is given in parentheses.

Genetic differentiation among populations (F_{DT}), was significant for the inversion polymorphism, *Est-1*, *Aldox*, *Est-2*, *Pep-1* and *Xdh*, but not significant for *Lap* and *Pep-2* (Table 2). However, F_{DT} for the inversion polymorphism was significantly greater than the average for electrophoretic loci ($P < 0.001$ for the bootstrap test evaluating the significance of the difference between F_{DT} estimates).

Est-2 and *Xdh* exhibited the largest F_{DT} values, which were not significantly different from each other ($P = 0.08$). Differentiation among populations was moderate for *Est-1*, *Pep-1* and *Aldox* (Table 2) and the corresponding F_{DT} 's were not significantly different from each other ($P = 0.18$), but significantly different from the average for *Est-2* and *Xdh* ($P = 0.04$).

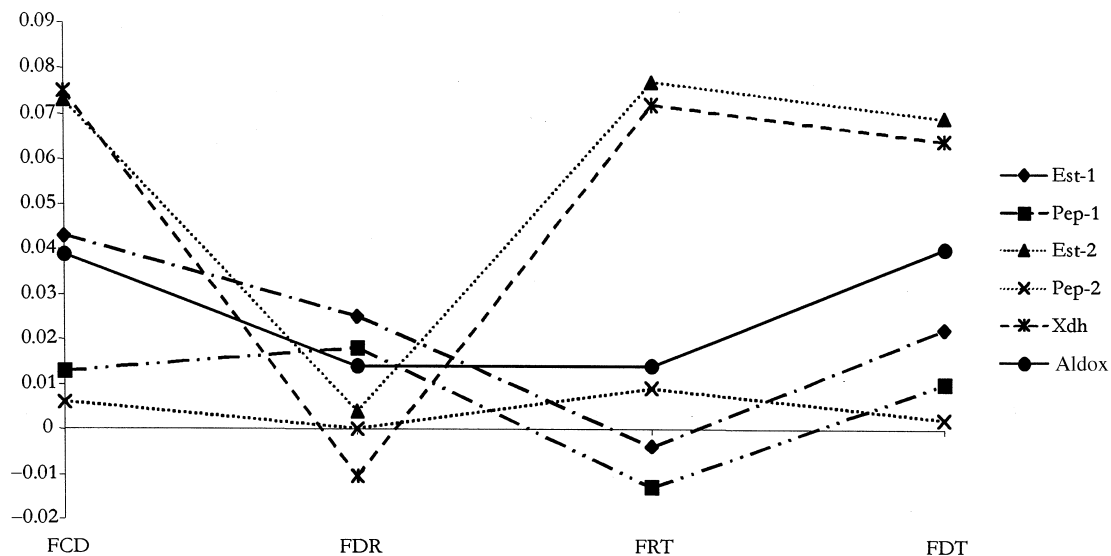
The hierarchical analysis of population structure for the inversion polymorphism revealed a strong regional pattern. Differentiation among regions within the total

(F_{RT}) was five times larger than among populations within regions (F_{DR}). Similar analyses for allozyme loci revealed at least two distinct patterns. First, *Est-2* and *Xdh* exhibited strong differentiation among regions. Second, differentiation among populations within regions was the main component of variation for the remaining loci (Table 2, Fig. 3).

The analysis of population structure considering arrangements within populations as the basic operational units of the hierarchy, revealed significant differences among arrangements for *Est-1*, *Est-2*, *Aldox* and *Xdh* as measured by the index F_{CD} (Table 2). However, bootstrap tests showed that F_{CD} estimates for *Xdh* and *Est-2* were significantly higher than for *Est-1* and *Aldox*.

These results are in agreement with expectations on the basis of the significant levels of linkage disequilibrium between these loci and inversions ($\chi^2_T = 46.46$, d.f. = 4, $P < 0.001$ for *Est-1*, $\chi^2_T = 69.00$, d.f. = 8, $P < 0.001$ for *Est-2*, $\chi^2_T = 25.10$, d.f. = 6, $P < 0.001$ for *Aldox*, and $\chi^2_T = 32.65$, d.f. = 6, $P < 0.001$ for *Xdh*). In general, the associations between the most frequent allozyme alleles of each locus and inversions were homogeneous across populations (Rodríguez, 1998).

However, bootstrap tests showed that differentiation among arrangements (F_{CD}) was lower for *Est-1* and *Aldox*, which map within the chromosomal segments rearranged by inversions, than for *Est-2* and *Xdh* (Table 2). Although this may not be unexpected for *Est-2*, which is tightly linked to the proximal break point of inversion 2*J* (Betrán *et al.*, 1995), the pattern observed for *Xdh* is curious as this locus is located outside the inverted segments (Ranz *et al.*, 1997). Moreover, Rodríguez (1998) estimated coefficients of linkage disequilibrium

**Fig. 3** Wright's *F*-statistics of the hierarchical analysis of population structure for seven allozyme loci (see text for an explanation of the meaning of the subindices).

(according to the formulae described in Weir, 1996) between each locus and inversions (D and D') and found for the particular case of Xdh that the most common allele, Xdh^b was positively and associated with $2J$ ($D = 0.05$, $D' = 0.27$, $\chi^2 = 19.22$, $P < 0.01$, d.f. = 1) and negatively with $2ST$ ($D = -0.06$, $D' = -0.21$, $\chi^2 = 24.7$, $P < 0.01$, d.f. = 1), whereas Xdh^c and Xdh^d were positively associated with $2ST$ ($D = 0.05$, $D' = 0.29$, $\chi^2 = 21.69$, $P < 0.01$ for Xdh^c and $D = 0.01$, $D' = 0.78$, $\chi^2 = 6.62$, $P < 0.01$ for Xdh^d , d.f. = 1 in both comparisons) and negatively associated with $2J$ (Xdh^c : $D = -0.04$, $D' = -0.30$, $\chi^2 = 16.28$, $P < 0.01$, and Xdh^d : $D = -0.01$, $D' = -0.74$, $\chi^2 = 3.96$, $P < 0.05$, d.f. = 1, in both cases).

Multiple-regression analyses of inversion frequencies on geographical variables (Table 3) accounted, on average, for more than 90% of among population variance. Frequency variation of $2ST$ was positively correlated with altitude and negatively correlated with latitude and longitude, whereas $2J$ and $2JZ^3$ exhibited the opposite trend. In addition, frequency variations of $2ST$ and $2J$ were significantly correlated with climatic variables (Table 3).

Variation of $Est-2$ allele frequencies were significantly correlated with geographical variables and, in this case, the multiple regression model accounted for more than

90% of total variance. The frequencies of $Est-2^a$ and $Est-2^d$ were negatively and positively correlated with latitude, respectively (Table 3). Among Xdh alleles, the frequency of Xdh^a was lower in lowland populations, Xdh^b and Xdh^c were positively and negatively associated with latitude, respectively, and Xdh^b was also positively correlated with mean annual atmospheric pressure (Table 3). Latitude was negatively and positively correlated with the frequencies of $Est-1^a$ and $Aldox^a$, respectively, and $Aldox^a$ was also positively correlated with temperature (Table 3). The $Pep-1$ and Lap did not show any significant association with geographical variables. Finally, multiple regressions of the frequency of $Pep-2^a$ on both geographical and climatic variables were significant; however, none of the partial regressions were significant.

From regression analysis of allele frequencies on environmental variables it is difficult to elucidate whether clines in allozyme frequencies result from hitchhiking or direct selection. This apparent problem can be solved by analysing the variation of allele frequencies within each arrangement, i.e. conditional allele frequencies (Voelker *et al.*, 1978). Conditional allele frequencies were calculated from the data obtained in the electrophoretic characterization of the F_1 progeny of each wild male. Conditional allele frequencies of $Est-1$ and $Aldox$

Table 3 Multiple regression analyses and partial regression coefficients of inversion or allele frequencies on geographical and climatic variables.

	Geographical variables						Climatic variables						
	Multiple regression			Partial regression coefficients			Multiple regression			Partial regression coefficients			
	R^2	$F(3,3)$	P	LAT	LON	ALT	R^2	$F(4,2)$	P	TEM	PRES	HUM	PPC
$2ST$	0.99	101.61	0.00	-1.2***	-1.16**	0.47*	0.97	17.31	0.05	0.22	-1.04	0.09	1.17
$2J$	0.96	22.58	0.01	1.18**	1.26*	-0.50	0.98	29.66	0.03	-0.19	0.90	0.36	-1.55
$2JZ^3$	0.93	13.86	0.03	1.19**	1.02*	-0.33	0.92	5.49	0.16	-0.41	0.85	-0.50	-0.71
$Est-2^a$	0.96	22.2	0.01	-0.68*	0.51	-0.13	0.97	14.64	0.06	0.37	-0.95	0.77	-0.42
$Est-2^b$	0.29	0.40	0.76	-0.63	-0.61	0.11	0.87	3.27	0.25	-1.28	-2.70	-2.89	-0.89
$Est-2^c$	0.81	4.21	0.13	0.63	-0.35	-0.03	0.94	7.47	0.12	0.39	2.03	-2.17	1.19
$Est-2^d$	0.91	9.90	0.05	0.98*	-0.13	0.27	0.91	5.26	0.17	-0.20	1.29	-0.63	-0.30
Xdh^a	0.89	8.01	0.06	-0.12	0.90	-1.60*	0.38	0.31	0.85	-0.04	-0.33	1.54	-0.78
Xdh^b	0.86	6.18	0.08	0.93*	0.21	-0.25	0.96	12.54	0.07	0.24	2.02*	-2.09	0.79
Xdh^c	0.95	8.45	0.06	-0.92*	-0.49	0.58	0.92	6.08	0.15	-0.26	-1.93	1.57	-0.39
$Est-1^a$	0.82	4.50	0.12	-1.01*	-0.03	-0.41	0.96	12.69	0.07	-0.19	-1.98	2.62	-0.94
$Est-1^b$	0.55	1.23	0.43	0.89	0.44	-0.12	0.60	0.75	0.64	-0.39	0.76	0.80	-0.06
$Est-1^c$	0.15	0.17	0.91	-0.39	-0.35	0.35	0.16	0.10	0.97	0.47	0.09	-0.14	0.19
$Aldox^a$	0.90	9.46	0.05	1.06*	0.10	0.21	0.98	22.93	0.04	-0.97*	0.16	0.35	-0.84
$Aldox^b$	0.78	3.51	0.16	-0.99	-0.28	0.08	0.78	1.87	0.38	0.50	-0.76	0.26	0.49
$Pep-2^a$	0.96	25.80	0.01	0.02	0.60	0.43	0.98	25.42	0.04	0.09	-0.36	-0.53	-0.11
$Pep-2^b$	0.52	1.06	0.48	0.09	-0.69	0.04	0.90	4.46	0.19	-0.77	-0.89	3.09	-1.89
$Pep-2^c$	0.69	2.25	0.26	-0.02	0.28	-1.07	0.51	0.52	0.74	0.65	1.47	-2.25	1.88

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

LAT: latitude; LON: longitude; ALT: altitude; TEM: mean annual temperature; PRES: mean annual atmospheric pressure; HUM: mean annual humidity; PPC: mean annual rainfall; P : associated probability.

Degrees of freedom of the multiple regressions are given in parentheses.

Table 4 Multiple regression analyses and partial regression coefficients of conditional allele frequencies on geographical and climatic variables.

		Geographical variables						Climatic variables						
		Multiple regression			Partial regression coefficients			Multiple regression			Partial regression coefficients			
		R^2	$F(3,3)$	P	LAT	LON	ALT	R^2	$F(4,2)$	P	TEM	PRES	HUM	PPC
2ST	<i>Est-2^a</i>	0.44	0.79	0.57	0.09	0.38	0.25	0.56	0.64	0.68	0.69	0.44	-0.31	-0.35
	<i>Est-2^b</i>	0.37	0.59	0.66	-0.62	0.18	-0.50	0.60	0.75	0.64	-0.82	-2.26	2.48	-0.90
	<i>Est-2^c</i>	0.41	0.69	0.62	-0.31	0.63	-1.05	0.12	0.07	0.99	0.02	-0.43	0.50	0.06
	<i>Est-2^d</i>	0.74	2.88	0.20	1.03	0.38	0.10	0.78	1.76	0.39	-0.10	1.20	-0.46	-0.69
2J	<i>Est-2^a</i>	0.77	3.45	0.17	-0.75	0.57	-0.60	0.82	2.35	0.32	1.12	0.51	-2.07	1.77
	<i>Est-2^b</i>	0.27	0.38	0.78	-0.62	-0.72	0.27	0.93	7.23	0.12	-1.39	-3.09*	4.60*	-2.27
	<i>Est-2^c</i>	0.85	5.72	0.09	0.99	-0.03	0.26	0.91	4.85	0.18	0.08	1.73	-1.41	0.14
	<i>Est-2^d</i>	0.92	11.79	0.04	1.06*	1.08*	-0.86	0.88	3.68	0.22	0.32	1.93	-1.46	0.02
2ST	<i>Xdh^a</i>	0.95	18.00	0.02	-0.97	-0.27	0.33	0.92	6.06	0.15	0.47	-0.91	0.43	0.21
	<i>Xdh^b</i>	0.00	0.004	1	-0.07	-0.1	-0.04	0.03	0.02	1	-0.30	-0.53	0.53	-0.19
	<i>Xdh^c</i>	0.06	0.06	0.98	-0.25	-0.04	0.08	0.07	0.04	0.99	0.12	0.58	-0.46	0.08
2J	<i>Xdh^a</i>	0.62	1.60	0.35	-0.4	1.46	-1.32	0.57	0.67	0.67	-0.62	-1.78	3.72	-2.71
	<i>Xdh^b</i>	0.96	25.22	0.01	1.14**	0.53	-0.24	0.99	66.63	0.01	-0.18	1.33*	-0.46	-0.64
	<i>Xdh^c</i>	0.78	3.65	0.16	-0.95	-1.21	0.96	0.87	3.37	0.24	0.84	0.43	-2.85	2.80

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

LAT: latitude; LON: longitude; ALT: altitude; TEM: mean annual temperature; PRES: mean annual atmospheric pressure; HUM: mean annual humidity; PPC: mean annual rainfall; P : associated probability.

Degrees of freedom of the multiple regressions are given in parentheses.

were not significantly correlated with environmental variables (data not shown), suggesting that the significant coefficients shown in Table 3 can be accounted for by hitchhiking with the inversion polymorphism. Contrarily, clines observed for *Est-2* alleles, particularly for *Est-2^d* within arrangement 2J, were stable after removing the effect of inversions (Table 4), significant associations between the conditional frequency of *Est-2^b* within 2J, with mean annual atmospheric pressure and humidity were also detected. In concordance with the trends depicted in Table 3, conditional frequency of *Xdh^b* within 2J varied clinally with latitude (Table 4). However, the observed associations between *Xdh^a* with altitude and *Xdh^b* with latitude and pressure (Table 3), respectively, were not significant when conditional frequencies were considered.

The association between allozyme variation and cactus hosts was crudely tested by regressing allele frequencies on the number of potential host plants present in each population. Regression analysis was only significant for *Est-2* alleles (*Est-2^a*: $\beta = 0.08$, $P = 0.01$; *Est-2^c*: $\beta = -0.07$, $P = 0.04$ and *Est-2^d*: $\beta = -0.05$, $P = 0.04$).

Discussion

Our comparative study revealed different patterns of geographical structure for chromosomal and allozyme variation. Population differentiation in inversion frequencies was an order of magnitude higher than the

average for electrophoretic loci. Thus, diversifying selection seems to be a plausible explanation for the inversion polymorphism, as random genetic drift cannot account for these contrasting patterns. The degree of structure depends on the genetic marker considered and usually inversions exhibited greater differentiation than other genetic markers such as recessive lethals and allozymes in almost all *Drosophila* species studied, with the exception of *D. pavani* (reviewed in Powell, 1997). Population structure for inversions seems to be the result of strong adaptive forces. Our hierarchical analysis of population structure showing greater differentiation among populations located in different phytogeographical regions also supports the adaptive explanation of geographical differentiation in arrangement frequencies in *D. buzzatii* (Hasson *et al.*, 1995). Furthermore, clines along geographical and climatic gradients for inversions suggest selective differentiation, an argument reinforced by concordant clines observed in areas recently colonized by *D. buzzatii* (Knibb & Barker, 1988; Fontdevila, 1989). Parallel patterns of geographical variation in inversion frequencies in different continents along geographical or climatic gradients have also been documented in several *Drosophila* species (reviewed in Krimbas & Powell, 1992; Powell, 1997), such as *D. melanogaster* (Lemeunier & Aulard, 1992) and *D. subobscura* (Prevosti *et al.*, 1990).

The degree of among population differentiation varied widely among allozyme loci and can be grouped in three different patterns of population structure. The first group

including *Lap* and *Pep-2* was characterized by negligible differentiation among populations. Low to moderate, but significant F_{DTs} , with higher differentiation among populations within regions distinguished the second group including *Pep-1*, *Est-1* and *Aldox*. The third group comprised of *Est-2* and *Xdh*, which showed relatively large F_{DTs} and greater differentiation among regions than among populations within regions. As similar levels of geographical variation are expected for neutral polymorphisms under drift and migration, these contrasting patterns suggest that certain loci may be subject to some form of natural selection. Thus, we can ask which one of the three patterns is not compatible with neutral expectations.

Two independent lines of evidence can help us to answer this question. It is generally accepted that mtDNA restriction fragment length polymorphisms may be considered selectively neutral and thus might be used to infer the evolutionary history of populations. Due to haploidy and uniparental inheritance, effective population size and genetic differentiation among populations for mitochondrial genes are expected to be four times lower and larger, respectively, than for nuclear genes (Crease *et al.*, 1990). The mtDNA sequence variation in *D. buzzatii* populations from Argentina was recently surveyed by Rossi *et al.* (1996), who showed that differentiation among populations, measured by the index G_{ST} (Takahata & Palumbi, 1985), was 0.18. Although inferences from comparisons between allozymes (present study) and mtDNA (Rossi *et al.*, 1996) may be compromised by the fact that the sets of populations studied in both works are only partially coincident, the ratio G_{ST}/F_{ST} for *Aldox* ($0.18/0.04 = 4.5$) is very close to the value expected under neutrality for both markers. On the other hand, for *Pep-2*, which showed the highest ratio (88.0), the presence of two alleles at intermediate frequencies throughout the surveyed area, suggests that balancing selection may be a plausible explanation for the absence of population structure in this locus. Although the ratios for *Est-2* (2.5) and *Xdh* (2.7) were greater than for the inversion polymorphism (0.71), population differentiation in these loci seems to be the consequence of adaptive factors. However, these conclusions should be taken with caution, as recent surveys of nucleotide variation in different mtDNA genes (Ballard & Kreitman, 1994; Rand *et al.*, 1994) cast some doubts on the selective neutrality of mtDNA variation.

Multiple regression analysis of allele frequencies on environmental variables gives also support to the hypothesis of selective differentiation for *Est-2* and *Xdh* and selective neutrality for *Aldox*. The observed significant associations between *Aldox* alleles and geographical and climatic variables were not stable when conditional allele frequencies within arrangements were used. Thus, hitchhiking seems to be the best explanation for the clines observed for *Aldox*, which is in linkage disequilibrium with the inversion system (Betrán *et al.*, 1995; Rodríguez, 1998; present paper). On the other hand, the

correlations of *Est-2* and *Xdh* allele frequencies with environmental variables were stable even after removing the effect of inversions, suggesting that the clines for these loci are independent of the clines observed for inversions and stressing the role of direct natural selection acting upon these loci or tightly linked regions. In addition, the patterns of variation detected for *Est-2* and *Xdh* detected by means of multiple-regression analysis were not consistent for different arrangements (Table 4). This is not unexpected in the case of *Est-2* because this locus is tightly linked to the inversion system (Schafer *et al.*, 1993; Betrán *et al.*, 1995). On the other hand, even though *Xdh* is outside the segments rearranged by inversions (Ranz *et al.*, 1997) we have detected significant gametic associations with the inversion polymorphism (Rodríguez, 1998, present paper), perhaps due to epistatic interactions with loci linked to the inversions (see below).

Significant spatial variation of *Est-1* and *Est-2* was also detected in a similar study performed in Australian populations of *D. buzzatii* (Knibb & Barker, 1988). Moreover, these authors showed that partial correlations between *Est-1* alleles and environmental variables can also be explained by hitchhiking. This suggests that the inversion polymorphism can account for the clines for *Est-1* but not for *Est-2*. Moreover, the patterns of variation observed in Argentina for *Est-2* paralleled those detected in recently colonized populations of Australia, suggesting that the clines developed after the introduction of *D. buzzatii* in this continent in a period no longer than 60 years. These coincident patterns of clinal variation in different continents point to strong selective forces shaping *Est-2* allele frequencies.

Est-2 is a α -carboxylesterase and it has been shown that, in *D. buzzatii*, it is mainly active in the gut, pointing to a role of *Est-2* in digestion or detoxification (East, 1982). Furthermore, carriers of *Est-2* alleles were shown to be differentially attracted to yeasts (Barker, 1990, 1994) and different cacti (Fernández Iriarte, 1999), which along with our present results point to a relationship with resource use. On the other hand, information about *Xdh* is more limited. This enzyme is related to purine metabolism and biochemical and genetic studies have shown in *D. melanogaster* that this enzyme is involved in complex physiological interactions with *Aldox*, which in *D. buzzatii* is in linkage disequilibrium with the inversion system, and *Pyridoxal oxydase* (O'Brien & McIntyre, 1978).

Although we cannot, at present, offer an explanation, the patterns of geographical variation observed for *Est-2* and *Xdh* resembled the inversion polymorphism, suggesting that epistatic interactions could be involved.

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