Temporal and spatial variation of inversion polymorphism in two natural populations of *Drosophila buzzatii*

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The inversion polymorphism of the cactophilic fly *Drosophila buzzatii* was studied in two natural populations. We assessed the temporal changes and microspatial population structure. We observed a significant increase in the frequency of arrangement 2J at the expense of 2ST in both populations. These gene arrangements appear to affect the life-history of flies differently. Environmental heterogeneity explains the karyotype coexistence in nature.

The analysis of population structure showed that differentiation of inversion frequencies among individual breeding sites, the rotting clacodes of *Opuntia vulgaris*, was highly significant. The karyotypic frequencies did not depart significantly from Hardy-Weinberg expectations, neither in individual rots nor in the total population. These results suggest that the observed population structure can be easily accounted by random genetic drift.

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Latitudinal and altitudinal clinal variation as well as seasonal and long-term changes have been observed for polymorphic paracentric inversions in the genus *Drosophila* (DOBZHANSKY 1970; ANDERSON et al. 1975; KRIMBAS and POWELL 1992). These observations have been explained first, so that the genetic changes are the response of the gene pool to environmental variation; and second, so that the pattern is due to the origin and spread of new adaptive karyotypes (ANDERSON et al. 1975). However, we know so little about the general ecology (natural breeding sites) of the most frequently studied species (e.g., *D. pseudoobscura* and *D. subobscura*) which makes testing between the two alternative hypotheses difficult.

D. buzzatii is a South American cactophilic species of the *repleta* group (WASSERMAN 1992), probably originated in the Argentinian Chaco (CARSON and WASSERMAN 1965; FONTDEVILA et al. 1982). *D. buzzatii* has successfully colonized the Mediterranean area (FONTDEVILA et al. 1981; FONTDEVILA 1989) and Australia (BARKER 1982) in historically recent times through human transport of its natural host plants.

In the New World populations inversion frequencies vary clinally along latitudinal and altitudinal gradients. This suggests that natural selection could have contributed to population structure (HASSON et al. 1995). It is, however, difficult to determine, whether the regional pattern can be solely attributed to environmental variables, since the utilization of different host plants in different phytogeographic regions also seems to have had a significant role (HASSON et al. 1995).

In Argentina, *D. buzzatii* breeds and feeds upon the necrotic tissues of several *Opuntia* cacti and columnar cactus species, such as *Trichocereus terschekii* and *Cereus validus* (HASSON et al. 1992). Since cactus species differ in their chemical composition (KIRCHER 1982), yeast diversity (STARMER et al. 1990) and other biologically significant variables for the flies as size, density and rot duration (HASSON et al. 1992), natural populations of *D. buzzatii* may experience the environment as spatially heterogeneous.

Drosophila species develop in discrete, ephemeral and heterogeneous habitats, and often the larvae are crowded. Under these ecological conditions, fast developing individuals and, consequently, with small adult body size (PARTRIDGE and FOWLER 1993; SANTOS et al. 1994) should be favored. Again, when breeding sites are scarce and larval density is low (competition is weak), individuals with longer developmental times and larger body sizes are expected to be favored (SEVENSTER and VAN ALPHEN 1993; Davis and HARDY 1994).

Moreover, in cactophilic *Drosophila*, the breeding site constitutes a discontinuous environment, and each rot pocket is colonized by a small number of adult flies (SANTOS et al. 1989; THOMAS and BARKER 1990; VILARDI et al. 1994; QUEZADA-DÍAZ et al. 1997). Under such ecological structure the distribution of genetic variation within and among breeding sites is expected to be driven mainly by random genetic drift.

Here we study temporal trends in inversion frequencies in two natural populations. They come from the southern margin of the distribution of *D. buzzatii*. In addition, we analyzed the effects of a rearing medium prepared with rotting tissues of *O. vulgaris* on life history traits of individuals with different second chromosome arrangements. Finally, we studied the microspatial population structure using the inversion polymorphism as a genetic marker.

MATERIALS AND METHODS

Otamendi and Arroyo Escobar are located 77 km and 40 km North-West of the city of Buenos Aires, in the Pampa Region. Both localities have a temperate climate with a wide amplitude between winter and summer temperatures. *Opuntia vulgaris* is the only host plant present in these localities.

Six samples of adult flies were obtained in 25 months in Otamendi and eight in Arroyo Escobar (HASSON et al. 1996) within a ten year period (Table 1a and 1b), by sweep netting on banana baits. Females were placed in individual vials and inversion frequencies were estimated from the inspection of the salivary gland chromosomes of one progeny larva from each vial.

In February 1992, inversion frequencies were also estimated in samples of third instar larvae collected from ten rotting clacodes of *O. vulgaris* collected in Otamendi. Salivary glands were dissected and processed for further cytological analysis. Karyotypic and inversion frequencies were estimated for each rot and for the total population.

Salivary gland chromosomes were prepared according to FONTDEVILA et al. (1981) and analyzed using the cytological maps and the descriptions of inversions reported in RUIZ et al. (1984). A killed yeast *Drosophila* medium was used in the experiments.

Temporal variations of inversion frequencies were investigated by means of correlation analysis with time (in months) and climatic data. Climatic variables including mean, maximum and minimum monthly temperature and total monthly rainfall, were obtained from Servicio Meteorologico Nacional.

As the samples varied widely, we used standardized frequency deviations. For each sample and arrangement these deviations were calculated using the formula: $(p_i - p_0)\ [\ (2N_i/p_0(1 - p_0)]^{1/2}$, where p_i is the

Table 1. *Relative frequencies of second chromosome arrangements in samples of D. buzzatii collected in Otamendi(a) and Arroyo Escobar(b).*
a)

Arrangement	10/91	02/92	10/92	02/93	04/93	11/93	r(1)	p(2)
ST	0.310	0.157	0.146	0.044	0.056	0.033	−0.86	0.027
J	0.554	0.701	0.701	0.758	0.717	0.818	0.84	0.036
JZ[3]	0.136	0.142	0.153	0.198	0.227	0.149	0.57	0.234
N	294	408	588	248	216	214		

b)

Arrangement	12/79	12/82	4/86	11/86	5/87	10/87	12/87	3/89	r(1)	p(2)
ST	0.310	0.146	0.156	0.201	0.116	0.124	0.078	0.109	−0.86	0.006
J	0.385	0.416	0.562	0.510	0.576	0.576	0.664	0.558	0.88	0.004
JZ[3]	0.296	0.416	0.265	0.284	0.308	0.297	0.258	0.332	−0.03	0.947
N	392	48	64	731	408	620	128	343		

N = number of chromosomes analyzed.
(1)correlation coefficient between standardized chromosomal inversion frequencies as a function of time (in months).
(2)significance level.

Table 2. *Correlation analysis coefficients between standardized frequencies of chromosomal inversion and climatic variables in Otamendi (1) and Arroyo Escobar (2) populations (Pop). All the correlations were not significant ($p > 0.05$).*

Arrangement	Pop	Mean-Temp	Max-Temp	Min-Temp	Rainfall
ST	1	−0.4816	−0.3942	−0.7451	0.7743
	2	0.1509	−0.0355	0.2359	0.4852
J	1	0.5437	0.4883	0.7793	−0.3812
	2	−0.0835	0.1934	−0.1579	−0.5536
JZ3	1	0.0998	−0.0464	0.3376	0.3098
	2	−0.1721	−0.2683	−0.2230	−0.0515

frequency of a certain arrangement in sample i and p_0 is the average frequency in the pooled samples and N_i is the number of individuals analyzed in each sample (CHRISTIANSEN et al. 1976).

In Otamendi, inversion frequencies were estimated, simultaneously, in samples of third instar larvae obtained from *O. vulgaris* rots and in the progeny of individual females. The comparison between these samples can be used to infer the operation of directional natural selection (RUIZ et al. 1986; HASSON et al. 1991). Comparisons were performed by means of contingency χ^2 tests and by the method devised by ANDERSON et al. (1979) using the following expression:

$$\Delta p/(\mathrm{Var}\Delta p)^{1/2}(\text{normal deviate}),$$

where

$$\mathrm{Var}\Delta p = p(1-p)(1/n_1 + 1/n_2),$$

$$p = \text{mean frequency} = {}^1/_2(p_1 + p_2);$$

n_1 and n_2 = sample sizes of each phase,

and

$$\Delta p = p_2 - p_1$$

where p_1 and p_2 correspond to the frequency of a certain arrangement in two different samples.

Life-history traits were studied in flies carrying different second chromosome arrangements, reared in conditions of optimal density (40 first instar larvae per vial) using a culture medium prepared with homogenates of *O. vulgaris* clacodes. Total viability was estimated as the proportion of individuals emerged, developmental time as the time elapsed since the sampling of first instar larvae until the emergence of adults. In addition, thorax length was scored in 50 individuals of each karyotypic class.

For the analysis of temporal and spatial population structure, fixation indices were estimated using the program FSTAT (GOUDET 1995). The significance of F_{IS} was tested according to LI and HORVITZ (1953): $\chi^2 = N\ F_{IS^2}$, and F_{ST}, which measures differentiation among samples, was evaluated using contingency χ^2 tests (NEI and CHESSER 1983). Finally, in order to determine whether differentiation has occurred at random, observed and expected correlations between frequencies of pairs of arrangements were compared according to SOKAL and ROHLF (1981). Expected correlations were estimated according to NEI (1965) and NEI and IMAIZUMI (1966) as

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

where $\bar{p}_m$ and $\bar{p}_n$ denote the mean frequencies of the m_{th} and n_{th} allele respectively.

RESULTS

Temporal patterns of inversion frequencies

Karyotypic frequencies in Otamendi and Arroyo Escobar estimated in each one of the temporal samples showed a close fit to Hardy-Weinberg expectations (data not shown). Temporal variation of second chromosome inversion frequencies in Otamendi revealed a decline of 2ST, as it is indicated by the significant and negative correlation coefficient (Table 1a). On the other hand, arrangement 2J exhibited the opposite trend. The frequency of arrangement $2JZ^3$ remained almost invariable with small random changes. Identical temporal trends were observed in Arroyo Escobar (Table 1b).

However, standardized inversion frequencies in both populations were not significantly correlated with any of the climatic variables analyzed (Table 2).

The comparison between samples of third instar larvae and adults collected in Otamendi yielded non-significant results ($\chi^2 = 3.34$, $df = 2$, $p = 0.19$) (Table 3). However, when we employed Δp test we detected significant changes. On one hand, the frequency of 2ST in adults was significantly higher than in third instar larvae, whereas 2J showed the opposite trend (Table 3). According to the model of selection components analysis (SCA) devised by RUIZ et al. (1986), differences in inversions frequencies between the third instar larval stage and the adult stage can be at-

tributed to the joint effect of differential fecundity and/or larval viability. Since two studies have shown that arrangement 2J increase fecundity (RUIZ et al. 1986; HASSON et al. 1991) this component of fitness seems to be the most plausible explanation for the present results.

Effects of Opuntia vulgaris rearing media on life-history associated with second chromosome karyotypes

Mean values of total viability, developmental time and thorax length of flies carrying different karyotypes are presented in Fig. 1. ST/* flies were more viable than J/* and JZ^3/*. Moreover J/* flies had slower developmental times and larger body sizes than JZ^3/* and ST/*.

Temporal and Microspatial Population Structure

Temporal variation of inversion frequencies was also analysed by means of Wright's F-statistics (Table 4). F_{IS} and F_{IT} indices were not significantly different from zero ($\chi^2 = 0.43$, df = 1, $p = 0.51$ and $\chi^2 = 3.09$, df = 3, $p = 0.38$, respectively). Thus, in each sample and in the total there was neither excess nor deficit of heterokaryotypes. This suggests that the population is mating at random (Table 4). Average F_{ST} was highly significant ($\chi^2 = 132.53$, df = 10, $p < 0.0001$,) as well as the contribution to differentiation of each individual arrangement (ST: $\chi^2 = 125.06$, $p < 0.0001$; J: $\chi^2 = 48.16$, $p < 0.0001$; JZ^3: $\chi^2 = 12.26$, $p < 0.05$; df = 5 in all cases). Confidence intervals (95 %) of F-statistics obtained by means of permutations tests yielded similar results (Table 4).

Absolute karyotypic frequencies in the samples of third instar larvae collected in each rotting clacode along with their corresponding F_{ISi} values are listed in Table 5. The proportion of negative and positive F_{ISi} values do not depart from the expected 1:1 ratio for samples taken from a population in Hardy-Weinberg proportions. Individual F_{ISi} values did not differ significantly from zero indicating that karyotypic frequencies did not depart from Hardy-Weinberg expectations in each individual rot. Similarly, average F_{IS} ($\chi^2 = 0.09$, df = 1, $p = 0.76$) and F_{IT} ($\chi^2 = 0.09$, df = 3, $p = 0.68$) were not significant. Among-rot differentiation was highly significant ($\chi^2 = 105.88$, df = 18, $p < 0.0001$) as well as the contribution to differentiation of the three arrangements (ST: $\chi^2 = 81.76$, $p < 0.0001$; J: $\chi^2 = 39.75$, $p < 0.0001$; JZ^3: $\chi^2 = 27.76$, $p < 0.001$; df = 9 in all cases). Permutation tests gave strong support to the results of χ^2 tests (Table 6). Finally, none of the comparisons between observed and expected correlations for pairs of arrangements were significant. This suggests that among rot differentiation took place mainly at random.

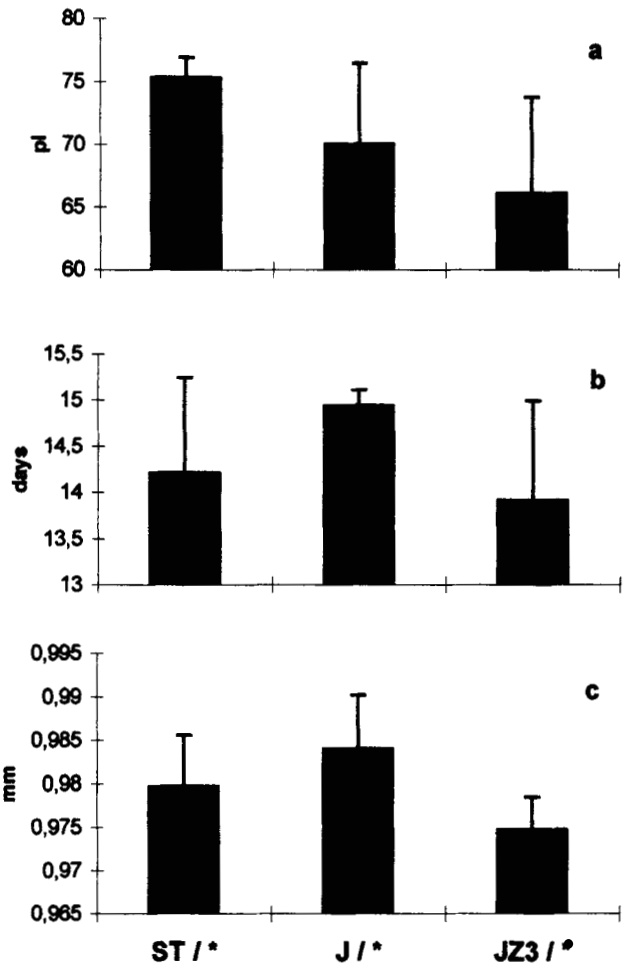

Fig. 1. a–c. Means values and deviations of life-history traits of flies carrying different karyotypes. * = any arrangement. **a** Total viability. **b** Developmental time. **c** Thorax length.

Table 3. *Relative frequencies of second chromosome arrangements in samples of adults and third-instar larvae of D. buzzatii collected in Otamendi in February 1992. Δp test for the significance of the changes of inversion frequencies between samples are also shown. In the last row the χ^2 (df = 2) values testing the goodness of fit to Hardy-Weinberg expectation are shown*

Arrangement	Adults	Larvae	Δp
ST	0.157	0.122	−0.034*
J	0.701	0.741	0.046**
JZ^3	0.142	0.137	−0.012
N	408	842	
χ^2	4.15	1.51	

*p = 0.054, **p < 0.05.
N = number of chromosomes analyzed.

DISCUSSION

The temporal pattern of variation of inversion frequencies in Otamendi and Arroyo Escobar may be considered to indicate directional selection. The trends observed could be the result, at least in part, of the response of the gene pool to new environmental conditions faced by *D. buzzatii* in populations located in the southern margin of its distribution. Climatic factors such as temperature and humidity may be responsible for the patterns of latitudinal and altitudinal clinal variation observed for 2ST and 2J (HASSON et al. 1995). The temporal shifts in the two populations were, however, not significatively correlated with climatic variables.

Direct evidence of the action of natural selection on the second chromosome polymorphism of *D. buzzatii* comes from the observed changes in inversion frequencies throughout the life-cycle (RUIZ et al. 1986; HASSON et al. 1991). These studies showed that an inversion may be adaptive in one stage but be disadvantageous in another. Therefore, long term temporal trends suggest that the potentially antagonistic effects of 2ST and 2J on different fitness components may be not completely balanced in Otamendi and Arroyo Escobar.

Another alternative explanation for the temporal trends is related to the trophic resource used by *D. buzzatii* (FONTDEVILA et al. 1981, 1982; RUIZ and FONTDEVILA 1985; RUIZ et al. 1986; HASSON et al. 1991, 1996; FERNÁNDEZ IRIARTE 1999) in Otamendi and Arroyo Escobar. Experiments with flies reared in media prepared with *O. vulgaris* rotting clacodes showed that 2J does not have a general advantage, since it lowers viability, increases developmental time and the average body size when compared to 2ST. These effects on life-history traits may indicate a trade-off between an early and a late fitness component (BETRÁN et al. 1998; FERNÁNDEZ IRIARTE 1999). Therefore, the net effect of inversions on fitness would be the result of particular features of the resources utilized by *D. buzzatii* in different populations. *O. vulgaris* may in fact, be a suboptimal resource. The viability of flies reared in media prepared with *O. vulgaris* was lower and developmental time was longer than in *O. ficus-indica* and *T. terschekii* (FERNÁNDEZ IRIARTE 1999). Moreover, natural rearing records showed that larval density in *O. vulgaris* is lower than in other *Opuntia* species, like *O. quimilo*, *O. sulphurea* and *O. ficus-indica*, which *D. buzzatii* use elsewhere (HASSON et al. 1992). Likewise, experimental populations of *D. buzzatii* fed with different parts of the same host plant, i.e. clacodes or fruits of *O. ficus-indica*, with different rates of decay, indicated that developmental time differences between arrangements may account for the shifts in inversion frequencies. On one hand, 2ST carriers, which develop faster than 2J, increased in frequency in population fed with fast decaying resources such as fruits, while 2J carriers were favored in population fed with slow rotting resources like clacodes (RUIZ and FONTDEVILA 1985). In Otamendi and Arroyo Escobar, larvae are, indeed, found only in *O. vulgaris* rotting clacodes.

Developmental time, a primary fitness component, may be a major factor determining not only the temporal patterns, but also the macrogeographic population structure (HASSON et al. 1995). Certain attributes of the larval substrate such as decaying rate (durability) may be more important than the specific host plant used by *D. buzzatii* in different populations. In Otamendi and Arroyo Escobar the lower larval density observed in *O. vulgaris* rots, with the consequent relaxation of competition, may favor slow developing individuals (SEVENSTER and VAN ALPHEN 1993) i.e. 2J carriers. This effect may be reinforced by the fact that another attribute of 2J carriers may be favored when breeding opportunities are scarce, since a large body size may be correlated with a greater dispersal ability.

Finally, the absence of a significant excess of heterokaryotypes in our analysis of the microspatial population structure in Otamendi agrees with the results previously reported in Arroyo Escobar (VILARDI et al. 1994). We have observed an excess of heterokaryotypes in populations from Chaco (HASSON 1988; FERNÁNDEZ IRIARTE 1999), the presumed

Table 4. *Analysis of population genetic structure by means of F-statistics for the adults samples collected in the population of Otamendi. Permutation test confidence intervals (95 %) are shown*

Arrangement	Average frequency	$F_{IS.k}$	$F_{IT.k}$	$F_{ST.k}$
ST	0.138	0.002	0.077	0.075
J	0.701	−0.023	0.005	0.027
JZ^3	0.162	−0.039	−0.034	0.005
Weighted average		−0.021	0.012	0.033*
Confidence interval		(−0.051 0.046)	(−0.047 0.048)	(−0.002 0.004)

*$p < 0.01$.

Table 5. *Absolute karyotypic frequencies and estimates of the fixation indices (F_{ISi}) for the second chromosome polymorphism of D. buzzatii in samples of third instar larvae taken from ten rotting clacodes of Opuntia vulgaris in Otamendi*

KARYOTYPE	ROT #									
	1	2	3	4	5	6	7	8	9	10
ST/ST		6				1				
ST/J	14	16	3	2	1	22	3	2	2	4
ST/JZ^3	7	4	1	1			1		1	1
J/J	107	13	4	6	4	35	36	13	7	3
J/JZ^3	37	2	4	1	4	3	8	5	5	5
JZ^3/JZ^3	3				1	1		1		1
Total	168	41	12	10	10	62	48	21	15	14
F_{ISi}	0.02	0.05	−0.20	0.07	0.02	−0.07	−0.02	0.08	−0.13	−0.16

Table 6. *Analysis of population genetic structure by means of F-statistics for the third-instar larvae samples collected on Opuntia vulgaris in the population of Otamendi. Permutation test confidence intervals (95 %) are shown*

Arrangement	Average frequency	$F_{IS.k}$	$F_{IT.k}$	$F_{ST.k}$
ST	0.123	−0.078	0.048	0.117
J	0.747	0.020	0.069	0.050
JZ^3	0.130	−0.017	0.014	0.031
Weighted average		−0.015	0.048	0.062*
Confidence intervals		(−0.086 0.082)	(−0.068 0.077)	(−0.008 0.014)

*$p < 0.01$.

area of origin of *D. buzzatii* and in a colonizing population from Spain (SANTOS et al. 1989), where larval density in each rot is on average higher. In populations where competition is more intense, density dependent regulation (soft selection) may favor heterokaryotypes with superior competition ability. This may be tested in laboratory experiments.

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