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The evolutionary history of *Drosophila buzzatti*. XXVI. Macrogeographic patterns of inversion polymorphism in New World populations

Esteban Hasson,[1,*] Constantina Rodríguez,[1], Juan J. Fanara,[1] Horacio Naveira,[2,†] Osvaldo A. Reig,[1] and Antonio Fontdevila[2]

[1]*GIBE, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria Pab. II,* (1428) *Buenos Aires, Argentina*
[2]*Departament de Genètica I de Microbiología, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain*

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Abstract

Inversion polymorphisms in the second and fourth chromosomes of the cactophilic *Drosophila buzzatti* in the native distribution range of the species are described. Over 5,000 flies from 26 localities were scored revealing interesting geographic structuring of arrangement frequencies. Multiple regression and partial correlation approaches showed that the frequencies of second and fourth chromosome arrangements vary clinically along latitudinal and altitudinal gradients and to a lesser extent with longitude. Although many non selective explanations can account for this pattern, its resemblance to the clinal pattern described in recently established Australian populations of *Drosophila buzzatii*, strongly suggests a selective explanation. Additionally, the correlated variation observed between the frequencies of arrangements 2*St* on the second chromosome and 4*St* on the fourth suggests a pattern of interchromosomal association, which, when considering the

* Present address: Department of Ecology & Evolution, State University of New York, Stony Brook, NY 11794-5245, USA.
† Present address: Departamento de Biologia, Universidad de Santiago de Compostela, Santiago de Compostela, Spain.

vast area surveyed, might be explained as the result of epistatic interactions. The analysis of population structure revealed a significant regional pattern, concordant with previously described phytogeographic regions. *F*-statistics showed that the patterns of variation were different not only between the second and fourth chromosomes, but also between second chromosome arrangements, suggesting that selective differentiation might have contributed to population structure.

Since *D. buzzatii* breeds and feeds on the decaying tissues of diverse cactus species present in different phytogeographic regions, and given that latitude and altitude are strong determinants of phytogeography, it is difficult to distinguish the underlying causes of the geographic patterns observed. However, inversion heterozygosity is not correlated with the diversity of potential cactus hosts.

The evidence presented suggests that differential selection may be the main cause for the population structure. It is also possible to conclude that the inversion polymorphism of *D. buzzatti* is flexible rather than rigid.

Introduction

Natural populations almost always display differences in allele frequencies from one geographic region to another. Such geographic population structure can have profound consequences on the evolutionary destiny of a species (Hartl and Clark, 1991) and it can be studied through genetic and ecological approaches. In the former, gene frequencies are estimated over the range of a species and the structuring can be quantified using Wright's *F*-statistics (Wright, 1978). The ecological approach involves measuring factors contributing to structure such as density and dispersal (Taylor and Powell, 1983).

Traditionally, polymorphic chromosomal inversions in the genus *Drosophila* have provided useful tools for directly examining the relationship between natural selection, population structure and evolution (Wright, 1978; Taylor and Powell, 1983; Craddock and Carson, 1989; Krimbas and Powell, 1992). Spatial and temporal patterns of variation have been interpreted as evidence for natural selection (Krimbas and Powell, 1992). In particular, clines, i.e. the gradual variation of gene frequencies along environmental gradients, are often cited as an argument in favor of selection. Clines have been described for the inversion polymorphism of a number of *Drosophila* species, such as *D. melanogaster* (reviewed in Lemeunier and Aulard, 1992), *D. pseudoobscura* (reviewed in Powell, 1992), *D. subobscura* (Prevosti et al., 1985), *D. robusta* (Etges, 1984; 1989), *D. flavopilosa* (Brncic, 1983).

Since the pioneering work of Wright and Dobzhansky (1947) trying to determine the nature of selection acting upon *D. pseudoobscura* inversion polymorphisms, two general alternative selective models have been invoked to explain their maintenance. The first proposes that under constant fitnesses, a stable polymorphism can be maintained if the heterozygote has a higher fitness than homozygotes. The alternative model assumes that relative fitness varies as a function of the frequencies of genotypes, as has been proposed for the extensive inversion polymorphism of *D.*

pseudoobscura (Salceda and Anderson, 1988). This model leads to a stable polymorphism when the fitness of a genotype increases as it becomes rare (Hedrick, 1983 pp. 218). However, varying selection on spatial as well as temporal scales can provide alternative explanations for the maintenance of genetic variation (Hedrick, 1983 pp. 201).

Studies on fruit fly species have suggested that chromosomal polymorphisms may be maintained via diverse causes in different species, introducing the concepts of so-called flexible and rigid polymorphisms (Dobzhansky, 1970). Species with flexible inversion polymorphisms, such as *D. flavopilosa*, show changing patterns of inversion frequencies in relation to environmental variables such as latitude and altitude, seasons of macrogeography (Brncic, 1983). On the other hand, species with rigid polymorphisms, such as *D. pavani*, do not show variation in inversion frequencies (Brncic, 1985). However, the actual agents responsible for the putative adaptive nature of chromosomal inversions are poorly understood. This is due, in part, to the difficulty in defining the natural habitats and the ecology of the three species most widely studied, e.g. *D. pseudooobscura* (Powell, 1992), *D. subobscura* (Krimbas, 1992) and *D. melanogaster* (Lemeunier and Aulard, 1992). Species whose breeding and feeding sites and other ecological features are amenable to ecogenetical experimentation, like mycophagous (Jaenike, 1990), flower breeding (Brncic, 1983) and cactophilic *Drosophila* (Wasserman, 1992), should certainly provide better models to explore the possible adaptive character of chromosomal polymorphisms.

The cactophilic *D. buzzatii* (buzzatii complex-mulleri subgroup, Ruiz and Wasserman, 1993) breeds and feeds on the decaying tissues of several species of Cactaceae in Argentina (Hasson et al., 1992), and has proven to be most rewarding for ecogenetical studies (Barker, 1990, Barker and East, 1980, Ruiz et al., 1986, Santos et al., 1989, Hasson et al., 1991). *D. buzzatii* is a South American species that has attained worldwide distribution, successfully colonizing the Mediterranean area (Carson and Wasserman, 1965; Fontdevila et al., 1981) and Australia (Barker, 1982) in historically recent times following its natural host plants. Carson and Wasserman (1965), Vilela et al. (1980) and Fontdevila et al. (1982) suggested the Argentinian Chaco as its most likely center of origin. Recent studies of inversion polymorphisms of *D. buzzatii* have investigated the correlation with several fitness components in two natural populations, one from Spain (Ruiz et al., 1986, Santos et al., 1989) and the other from Argentina (Hasson et al., 1991). Studies surveying chromosomal variation in South America (Fontdevila et al., 1982; Barker et al., 1985) have included too few populations to properly characterize population structure. A detailed description of population structure in the ancestral South American populations is essential to interpret the patterns observed in the colonized areas (Fontdevila et al., 1981; Knibb and Barker, 1988). In the present paper, we report results of an extensive survey, including collections in twelve Argentinian localities sampled for the first time that allowed us to analyze the macrogeographic pattern of variation of the inversion polymorphism of *D. buzzatii* in its original area of distribution.

Materials and methods

Localities sampled

D. buzzatii ranges from 15 to 35° S latitude throughout Brazil, Bolivia, Paraguay, Uruguay and Argentina. Several cactus species are exploited by *D. buzzatii*. In the surveyed area the endemic *Opuntia quimilo*, *O. vulgaris*, *O. maxima*, *O. cordobensis*, *O pampeana*, *O. sulphurea*, and the introduced *O. ficus-indica* among Opuntioids, and *Cereus validus*, *Trichocereus terschekii*, *T. pasacana*, *Stetsonia corynne* among Cereoid *species*, can serve as breeding sites (Tab. 1).

The geographic location of all populations sampled for the present study are shown in Fig. 1. Some of these localities were reported in previous studies by Fontdevila et al. (1982), Ruiz (1982) and Barker et al. (1985). A detailed description of the localities including geographic coordinates, elevation and cactus species present is shown in Table 1. In addition, the assignment of each sampling locality

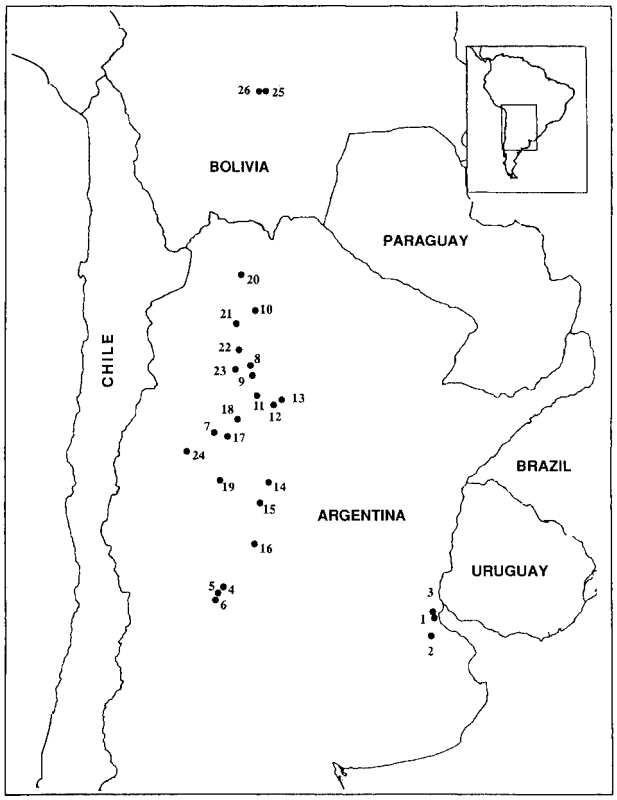

Fig. 1. Geographic location of the *Drosophila buzzatii* populations included in the present study. See Table 1 as a reference key to populations.

Table 1. Localities included in the present study are shown along with their geographical coordinates, altitude above sea level, and the phytogeographic region and subdivision to which they belong (according to Cabrera, 1976). The most abundant cactus species are also indicated for each locality.

Locality	Region	Subdivision	Latitude	Longitude	Altitude	Cactus species
1. Arroyo Escobar	Pampa	Meridional	34.4	58.7	5	O.v.
2. Moreno	Pampa	Meridional	34.7	58.8	20	O.f-i.
3. Otamendi	Pampa	Meridional	34.3	58.8	10	O.v.
4. San Luis Sierra	Espinal	Meridional	33.2	66.2	709	T.c., O.p.
5. San Luis Oeste	Espinal	Meridional	33.3	66.5	566	O.f-i.
6. El Puesto	Espinal	Meridional	33.4	66.6	450	O.f-i.
7. Mazan	Southern Monte	Monte	28.8	66.4	1300	T.t, O.s.
8. Vipos	Chaco Serrano	Central Chaco	26.5	65.4	786	T.t., C.v., O.q.
9. El Cadillal	Chaco Serrano	Central Chaco	25.5	65.2	700	T.t., C.v., O.q.
10. Guemes	Chaco Serrano	Central Chaco	24.7	65.1	734	S.c., C.v., O.q.
11. Rio Hondo	Western Chaco	Central Chaco	27.5	65.9	290	S.c., C.v., O.q., O.f-i., O.p.
12. Santiago de Estero	Western Chaco	Central Chaco	27.9	64.3	189	S.c., C.v., O.q., O.p.
13. San Lorenzo	Western Chaco	Central Chaco	28.0	64.5	278	S.c., C.v., O.q., O.p.
14. Dean Funes	Western Chaco	Central Chaco	30.4	62.7	689	O.q., O.f-i.
15. Diquecito	Southern Chaco	Central Chaco	31.4	64.4	550	O.c.
16. Villa Dolores	Southern Chaco	Central Chaco	32.0	65.2	521	O.p., C.sp.
17. Chumbicha	Transitional Chaco	Central Chaco	28.8	66.3	421	T.t., S.c., C.v., O.q.
18. Palo Labrado	Transitional Chaco	Central Chaco	28.4	65.6	700	T.t., S.c., C.v., O.q.
19. Patquia	Transitional Chaco	Central Chaco	30.1	66.8	300	T.t., S.c., C.v., O.q.
20. Tilcara	Prepuna	Prepuna	23.6	64.5	2200	T.p., O.s.
21. Cachi	Northern Monte	Monte	25.1	65.2	2280	T.t., O.s.
22. Cafayate	Northern Monte	Monte	26.1	66.0	1660	T.t., O.s.
23. Quilmes	Northern Monte	Monte	26.6	65.9	2000	T.t., O.s.
24. Famatina	Southern Monte	Monte	29.0	67.2	1600	T.t., O.s
25. Comarapa	Bolivian Chaco	Bolivian Chaco	18.1	64.5	3000	C.d., C.c., N.h., R.t., O.s.
26. Los Negros	Bolivian Chaco	Bolivian Chaco	18.1	64.6	3000	C.d., C.c., N.h., R.t., O.s.

Key to Cactus Species: O.v = *Opuntia vulgaris*, O.q. = *O. quimilo*, O.s. = *O. sulphurea*, O.c. = *O. cordobensis*, O.p. = *O. pampeana*, O.g. = *O. glomerata*, O.f-i. = O. ficus-indica, S.c. = Stetsonia corynne, C.v. = Cereus validus, C.sp. = Cereus sp., C.d. = C. dayamii, C.c. = *C. comarapanus*, T.c. = *Trichocereus candicans*, T.t. = *T. terschekii*, T.p. = *T. pasacana*, N.h. = *Neocardenasia herzogiana*, R.t. = *Roseocereus tephracactus*.

to Phytogeographic Regions and Subdivisions, based on the classification of Cabrera (1976) is also shown in Table 1.

Collecting and cytological methods

Adult flies were collected by net sweeping on banana baits. Females were placed individually in vials containing a modified formula of David's killed yeast medium (David, 1962). Inversion frequencies were estimated through the analysis of one larval progeny from each isofemale line, a common procedure for estimating gene frequencies (Heed and Carson, 1983). Polytene chromosome preparations were obtained and scored according to Fontdevila et al. (1981).

Data analysis

The geographic pattern of the inversion polymorphism was analyzed by means of Wright's hierarchical F-statistics (Wright, 1978) and multiple regression and partial correlation analysis.

The calculation of Wright's fixation indices requires the estimation of the actual variance of gene frequencies (σ_T^2) divided by the limiting variance, $\bar{q}_T(1-\bar{q}_T)$. Later on, it was also shown that F statistics can be defined in terms of observed and expected heterozygosities (Nei, 1987 pp. 160) for the analysis of mating structure and in terms of gene diversity to analyze gene frequency variation, in the case of hierarchically structured populations (Nei, 1973). F-statistics are commonly used as a measure of population subdivision. Based on equilibrium expectations, derived from theoretical models, these indices can also be used as an indirect estimate of gene flow, as the absolute number of individuals exchanged per generation between populations (Nm, where N is the effective population size and m is the migration rate per generation) (Wright, 1969).

Although the hierarchical structure is used for analyzing population structure arbitrary generally, in the present case populations were grouped according to biological criteria. All *D. buzzatii* populations (D for demes) analyzed are within the limits of the Chaco Phytogeographic Dominium (Cabrera, 1976). The Chaco Dominium can be further subdivided into Phytogeographic Provinces (R for regions) (Cabrera, 1976), which in turn, were considered the next level of the hierarchy. Thus, all populations were grouped according to the Phytogeographic Province where they are located. As shown in Table 1, we have considered the following regions: I. Pampa, including populations 1–3; II. Espinal, populations 4–6; III. Southern Monte, populations 7 and 24; IV. Northern Monte, 21–23; V. Prepuna, 20 and VI. Bolivian Chaco, 25 and 26. The remaining populations, included within the limits of the Chaco Phytogeographic Province, were assigned to the recognized districts of this diverse region (Cabrera, 1976): VII. Western Chaco, populations 11–14; VIII. Southern Chaco, 15–16; IX. Chaco Serrano, 8–10 and X. Transitional Chaco, 17–19 (Table 1). These regions were further grouped into five

subdivisions. A. – region II, Espinal, is considered as an impoverished Chaco and accordingly as a transition between the latter and the Pampa. Therefore, these two regions were grouped in the same subdivision. B. Central Chaco included regions VII–X according to the criterion mentioned above. C. Monte, regions III and IV correspond to different areas of the Monte Phytogeographic Province. D. Prepuna, only one region (V) was included in this subdivision. E. region VI, based on the differences of cactus diversity in the Bolivian Chaco populations, they were grouped in an unique subdivision.

F-statistics were estimated according to Wright (1978) using step Wright '78 of the program Biosys-1 (Swofford and Selander, 1981). The array of fixation indices are related through Wright's general equation:

$$1 - F_{DT} = (1 - F_{DR})(1 - F_{RS})(1 - F_{ST})$$

Fixation indices measure the degree of differentiation among different levels of the hierarchy relative to a higher level. For example, F_{DT} measures differentiation among all populations, F_{DR} among populations within regions; F_{RS}, among regions within subdivisions and F_{ST} among subdivisions.

In order to test for the significance of the differences of inversion frequencies among regions a nested ANOVA with subdivisions (random factor) and regions nested in subdivisions (random) as main effects was performed as suggested by Weir (1989, pp 158–159). This methodology generates estimates of the different components of variance. Prior to the ANOVA, inversion frequencies were normalized using an angular transformation (arcsin $(p_{ij})^{1/2}$, where p_{ij} is the frequency of arrangement i in population j).

In addition, the association between the three most common second chromosome arrangements (2standard-*St*-, 2*j* and 2*jz*3) and the standard arrangement of chromosome 4 (4*St*) with geographic variables (latitude, longitude and altitude) was tested by means of multiple regression. However, as the geographic variables are themselves correlated over the collecting sites (latitude-elevation $r = -0.807$, latitude-longitude $r = -0.326$ and elevation-longitude $r = 0.305$), partial correlations were also calculated to give better estimates of the associations between the inversion frequencies and the geographic variables. Again, the same angular transformation of inversion frequencies was employed. Expected heterozygosities (H) were calculated for the second and fourth chromosome polymorphisms for each population. Their association with the geographic variables was assessed by using the same methods described above with arcsin ($H^{1/2}$) transformed values.

Results

Inversion polymorphism of D. buzzatii

The frequencies of arrangements and the expected heterozygosities for both the second and fourth chromosomes in each locality are given in Table 2. They comprise 12 populations reported here for the first time and 14 samples from

Table 2. Frequency distribution of the chromosomal polymorphism and expected heterozygosities (H) in South American populations of *Drosophila buzzatii* sampled up to the present report. N = number of chromosomes analyzed.

	Chromosome 2										Chromosome 4		
Locality	N	St	j	jz^3	y^3	jq^7	jc^9	r^9	js^9	H	St	s	H
1. Arroyo Escobar[4]	620	0.124	0.576	0.297	–	0.003	–	–	–	0.565	0.994	0.006	0.012
2. Moreno[4]	130	0.162	0.523	0.315	–	–	–	–	–	0.601	0.992	0.008	0.018
3. Otamendi[4]	294	0.310	0.550	0.136	–	0.003	–	–	–	0.583	0.969	0.031	0.061
4. San Luis Sierra[1]	198	0.323	0.515	0.091	0.040	–	0.025	–	–	0.620	0.990	0.010	0.019
5. San Luis Oeste[1]	36	0.333	0.472	0.194	–	–	–	–	–	0.629	1.000	–	0.000
6. El Puesto[1]	76	0.303	0.526	0.079	–	–	–	–	–	0.625	0.905	0.095	0.172
7. Mazan[4]	12	0.333	0.667	–	–	–	–	–	–	0.444	1.000	–	0.000
8. Vipos[4]	304	0.256	0.724	0.010	0.010	–	–	–	–	0.411	1.000	–	0.000
9. El Cadillal[4]	718	0.333	0.634	0.033	–	–	–	–	–	0.486	1.000	–	0.000
10. Guemes[4]	140	0.336	0.664	–	–	–	–	–	–	0.446	1.000	–	0.000
11. Rio Hondo[4]	956	0.582	0.398	0.020	–	–	–	–	–	0.502	1.000	–	0.000
12. Santiago de Estero[3]	42	0.429	0.547	0.024	–	–	–	–	–	0.516	1.000	–	0.000
13. San Lorenzo[1]	346	0.538	0.413	0.049	–	–	–	–	–	0.538	0.997	0.003	0.006
14. Dean Funes[1]	197	0.604	0.391	0.005	–	–	–	–	–	0.482	1.000	–	0.000
15. Diquecito[1]	54	0.389	0.593	0.018	–	–	–	–	–	0.497	1.000	–	0.000
16. Villa Dolores[1]	43	0.279	0.605	0.116	–	–	–	–	–	0.543	0.977	0.023	0.045
17. Chumbicha[4]	86	0.523	0.430	0.047	–	–	–	–	–	0.539	1.000	–	0.000
16. Palo Labrado[4]	176	0.449	0.545	0.006	–	–	–	–	–	0.501	1.000	–	0.000
19. Patquia[3]	18	0.389	0.611	–	–	–	–	–	–	0.475	0.944	0.056	0.106
20. Tilcara[4]	128	0.064	0.766	0.081	–	–	0.089	–	–	0.395	0.602	0.398	0.479
21. Cachi[4]	126	–	1.000	–	–	–	–	–	–	0.000	0.057	0.943	0.108
22. Cafayate[4]	186	0.048	0.952	–	–	–	–	–	–	0.091	0.379	0.621	0.471
23. Quilmes[4]	56	0.018	0.982	–	–	–	–	–	–	0.035	0.482	0.518	0.499
24. Famatina[3]	22	0.318	0.545	0.136	–	–	–	–	–	0.583	0.636	0.364	0.463
25. Comarapa[2]	26	0.115	0.808	–	–	–	–	0.038	0.038	0.331	1.000	–	0.000
26. Los Negros[2]	180	0.250	0.722	–	–	–	–	0.011	0.017	0.416	1.000	–	0.000

1: Fontdevila et al., 1982, 2: Ruiz (1982), 3: Barker et al., 1985, 4: present paper.

previous studies (Fontdevila et al., 1982; Barker et al., 1985). Arrangements $2St$ and $2j$ are the most ubiquitous, whereas $2jz^3$ shows a more limited distribution. Arrangements $2y^3$, $2jc^9$ and $2jq^7$ are very rare and are present in at least one population.

There is broad variation in arrangement frequencies among populations. Populations tend to be polymorphic for the second chromosome, except in Northern Monte (localities 21–23) where the $2j$ arrangement is almost fixed. Interestingly, these same localities show the highest degree of polymorphism for the fourth chromosome. Rare endemics (Ruiz et al., 1985) are found at moderately low frequencies in more than one locality. One rare endemic, $2jq^7$, present at moderately high frequencies in several localities of the colonized areas in the Old World was detected in two populations (1 and 3) of the Pampean region. There are no obvious temporal shifts in arrangement frequencies with respect to previous reports (Fontdevila et al., 1981; Barker et al., 1985). However, significant changes exist in Arroyo Escobar when compared with data previously reported by Fontdevila et al. (1982). In this locality a continuous ten year decline for $2St$ has been observed (Hasson, 1988).

No excess of heterozygotes was observed. Observed karyotypic frequencies did not depart significantly from Hardy-Weinberg expectations, neither in the populations reported in this paper nor in those previously reported by Fontdevila et al. (1982) and Barker et al. (1985). However, heterozygosities also showed extensive variation among populations. Second chromosome heterozygosity was relatively high in lowland populations and extremely reduced in almost all populations at higher altitudes (Northern Monte and Prepuna). The opposite picture was observed for fourth chromosome heterozygosity, while lowland populations tended to be almost monomorphic, Monte and Prepuna populations were highly polymorphic.

Macrogeographic patterns of chromosomal polymorphism

The results of the hierarchical F-statistics analysis are shown in Table 3. The patterns of variation detected for chromosome 2 and 4 were clearly different. These differences are not only evident when total variation is compared (Tab. 3 F_{DT} column), but also when comparing the pattern in which total variation is partitioned among the different levels of the hierarchy. On one hand chromosome 2 seems to vary at a regional scale, as suggested by the large contribution of F_{RS} to differentiation among regions within the total, and on the other, chromosome 4 seems to vary at a larger scale. Another important feature detected with F-statistics consisted of the differences in the pattern of variation detected among second chromosome arrangements (Tab. 3).

The ANOVAs performed confirmed the trends revealed by F-statistics. The among-region within-subdivisions component of variation was not only significant in all cases ($2St : F_{5,16} = 5.0$, $p = 0.006$; $2j : F = 9.7$, $p = 0.002$; $2jz^3 : F = 3.5$, $p = 0.025$ and $4St : F = 6.1$, $p = 0.002$) but it was also the most important component of variation for $2St$ (47%) and $2j$ (68%) when compared to $2jz^3$ (29%) and $4St$ (31%).

Table 3. *F*-statistics for the hierarchical analysis for chromosome 2 and chromosome 4, and for each of the three more common second chromosome arrangements. Weighted average *F*-statistics for the second chromosome and for the total were estimated according to Wright (1978) using the program Biosys (see Materials and methods for details). *D* corresponds to the lowest level of the hierarchy demes or local populations, *R* for regions, *S* for subdivisions and *T* for total.

	FDR	*FRS*	*FST*	*FDT*
Chromosome 2	0.015	0.077	0.030	0.118
2*St*	0.015	0.085	0.031	0.127
2*j*	0.008	0.087	0.022	0.114
$2jz^3$	0.043	0.026	0.054	0.118
Chromosome 4	0.179	0.259	0.251	0.544
Average	0.048	0.120	0.093	0.240

Clinal patterns of variation of inversion frequencies

Second chromosome arrangements showed different degress of association with geographic variables (Tab. 4). About 54% of the among-populations variance of 2*St* could be explained by a significant multiple regression model, indicating that the frequency of this arrangement decreased significantly with increasing altitude and latitude (Tab. 4). Multiple regression of 2*j* was also significant, accounting for

Table 4. Multiple regression and partial correlation coefficients of arcsin $(p)^{1/2}$ transformed frequencies of the three most common second chromosome arrangements and arrangement 4*St* of *D. buzzatii* on latitude (Southern), altitude and longitude (Western). The results of a similar analysis of arcsin $(p)1/2$ transformed expected heterozygosity (*H*) for both second and chromosome polymorphisms are also shown. The *F*-ratio and the correlation coefficient for the multiple regression model are shown along with the regression coefficients and partial correlations (on each variable when controlling for the effects of the other two) of each inversion on latitude (degrees), elevation (in meters) and longitude (degrees).

	Chromosome 2				Chromosome 4	
	St	*j*	jz^3	*H*	*St*	*H*
Multiple regression						
R^2	0.539	0.483	0.578	0.372	0.376	0.331
F	8.59**	6.85**	10.03**	4.35*	4.41*	3.62*
Regression coefficients						
Latitude	−0.027*	0.009	0.030**	−0.002	−0.54*	0.048*
Elevation	-3×10^{-4}***	2×10^{-4}**	4×10^{-5}	-2×10^{-4}*	-4×10^{-4}*	3×10^{-4}***
Longitude	0.026+	−0.004	−0.026*	-4×10^{-4}	−0.015	0.017
Partial correlation						
Latitude	−0.147*	0.146	0.543**	0.026	−0.444*	0.483*
Elevation	−0.692***	0.569**	0.159	=0.428*	−0.590**	0.554**
Longitude	0.358*	−0.062	−0.438	−0.004	−0.117	0.170

+ $= 0.05 < p < 0.10$; * $= 0.01 < p < 0.05$; ** $= 0.001 < p < 0.01$; *** $= p < 0.001$.

48% of the total variance. In this case, altitude was the only significant variable accounted for by the model (Tab. 4). The analysis of frequency variation of inversion $2jz^3$ yielded highly significant results and the multiple regression model accounted for 58% of the total among population variance. The frequency of this arrangement increased at higher latitudes and towards the east among localities coded by longitude (Tab. 4). Fourth chromosome polymorphism also showed significant associations with geographic variables. A multiple regression model accounting for 38% of the total variance revealed that the frequency of the standard arrangement decreased significantly with increasing latitude and at higher altitudes (Tab. 4).

These patterns of variation were confirmed, with only one exception, by partial correlation analysis (Tab. 4). This methodology allows better estimates of the association between inversion frequencies and geographic variables by controlling for the effects of correlated variables. The exception was the non significant correlation between the frequency of $2jz^3$ and longitude.

Another interesting feature of the clinal variation detected was the close resemblance of the patterns observed for arrangements $2St$ and $4St$. The frequencies of these two arrangements were significantly correlated ($r = 0.76$, $p < 0.0001$).

Either second and fourth chromosome heterozygosities showed significant but opposite patterns of variation with respect to altitude. Second chromosome heterozygosity was negatively correlated with altitude and fourth chromosome heterozygosity was positively correlated with altitude and latitude (Tab. 4).

Discussion

Population genetic structure arises as a consequence of differential selection, drift, and migration. Our study shows that the inversion polymorphism of *D. buzzatii* is geographically structured in its native habitat in the southern arid and semiarid regions of South America. The observed regional pattern of inversion frequencies concordant with phytogeographic regions and the significant associations of inversion frequencies with geographic variables suggest that natural selection could have contributed to population structure.

Evidence supporting the interpretation of the observed patterns as the result of differential selection in different populations comes from previous work in two natural populations of *D. buzzatii* (Ruiz et al., 1986; Hasson et al., 1991). In these studies a consistent relationship between inversion polymorphism and several fitness components has been demonstrated by means of selection component analysis.

Since *D. buzzatii* is a widely distributed species it is possible to look for common patterns of variation in different areas. The three most common second chromosome arrangements in Argentina are also found in Australian populations. After introduction, the flies rapidly expanded over the vast area occupied by the introduced *Opuntia* species, spanning a total 20° in latitude (Knibb et al., 1987). Knibb and Barker (1988) reported that the spatial frequency variation observed for arrangements $2St$ and $2jz^3$ are negatively and positively correlated with latitude,

respectively. These clinal patterns are strikingly similar to our results. Furthermore, the frequencies of arrangements 2*St* and 2*j* are positively and negatively correlated with temperature (Knibb and Barker, 1988). These correlations coincide with the patterns expected from the clines observed in South America, provided that temperature is negatively correlated with latitude and altitude. The occurrence of such parallel clines in different continents strongly supports our hypothesis of selective differentiation.

A similar picture was observed in the Palearctic *D. subobscura* (reviewed in Krimbas, 1992). Latitudinal clines were described for the inversion polymorphism by the earlier *D. subobscura* workers in its native area of distribution (Europe and Northern Africa) (Prevosti et al., 1985) and, since the finding of concident patterns in South and North America, two recent independent colonizations, it is widely recognized that such a population structure arose as an adaptive response of the inversion system (Prevosti et al., 1985, 1988, 1990).

D. melanogaster is a cosmopolitan species highly polymorphic for paracentric inversions that also shows parallel clines for the four cosmopolitan inversions in each major autosomal arm in Australia, Asia and North America (reviewed in Lemeunier and Aulard, 1992, and references therein). Moreover, in some cases the data are strikingly coincident suggesting that those patterns are the result of differential selection along environmental gradients.

Along the clines observed in South American populations of *D. buzzatii*, the standard arrangements of both polymorphic chromosomes (2*St* and 4*St*) show similar patterns of variation with geographic variables, and their frequencies are significantly correlated over the vast area surveyed. Interestingly, they each represent an ancestral karyotype (Wasserman, 1992). Two possible explanations can account for this pattern: 1) both arrangements are responding to the same selective agents and/or 2) these associations are the result of epistatic selection. This type of significant interchromosomal association has been reported in *D. melanogaster* (Lemeunier and Aulard, 1992) and *D. robusta* (Etges, 1984) and its stability over time was taken as evidence for epistasis.

Since historical events, caused by fluctuations of effective population size and migration, should have the same effect on the among population differentiation at all loci, the differences in the patterns of variation between chromosomes and among second chromosome arrangements revealed by *F*-statistics give also support to the hypothesis of selective differentiation. Similar patterns have been described in *D. pseudoobscura* (Wright, 1978; Taylor and Powell, 1983) and *D. subobscura* (Ferrari and Taylor, 1981).

The comparison of the patterns of among population differentiation between the ancestral and the recently colonized areas shows that it is higher in South America (Fontdevila, 1991). Likewise, Fontdevila et al. (1981) and Knibb et al. (1987) reported that in Old World and Australian populations, respectively, the levels of inversion polymorphism in colonized areas were lower than in South America because of the loss of low frequency arrangements, due to founder effects. *F*-statistics show that most interpopulation differentiation in the Old World can be accounted for by demes inside small regions, and very little by regions within geographical areas (Fontdevila 1991), in sharp contrast with the pattern observed in South America.

These observations support the previous suggestion by Fontdevila (1991) that the simplest explanation for the latter would need to invoke ecological gradients, whereas historical events could account for differentiation in the Old World.

The causal factors underlying the clinal and the regional patterns of variation of the inversion polymorphism of *D. buzzatii* are not totally clear. Since latitude and altitude are strong determinants of climate and phytogeography is correlated with climate, it is difficult to determine whether the regional pattern can be solely attributed to environmental variables. The utilization by *D. buzzatii* of different host cactus species in different regions could be a possible explanation for the population structure, yet average heterozygosity is not correlated with the number of potential host plants ($r = -0.31$, $0.10 < p < 0.25$).

The present results show that the inversion polymorphism of *D. buzzatii* is not of the rigid type as had previously been suggested (Carson, 1965; Sperlich and Pfriem, 1986). However, rigid and flexible polymorphisms refer to ancient concepts and there is a further need to address the direct causes underlying such different responses to environmental factors.

Recently, several enzyme loci has been mapped with respect to the inversion system of *D. buzzatii* (Schafer et al., 1993) and it was also shown that certain loci are in linkage disequilibrium with the inversions (Knibb et al., 1987).

Recent advances in molecular biology allow us to study the pattern of DNA sequence variation, which can be used to infer evolutionary forces acting on specific polymorphisms (e.g. Hudson, 1990). A survey of nucleotide variation for genes associated with the inversions would provide a useful picture for inferring the mechanisms causing the patterns observed in *D. buzzatii.*

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