The Evolutionary History of *Drosophila buzzatii*. XXX. Mitochondrial DNA Polymorphism in Original and Colonizing Populations

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Both original and colonizer populations of *Drosophila buzzatii* have been analyzed for mtDNA restriction polymorphisms. Most of the mtDNA nucleotide variation in original populations of NW Argentina can be explained by intrapopulation diversity and only a small fraction can be accounted for by between-population diversity. Similar results are obtained using either the estimated number of nucleotide substitutions per site or considering each restriction site as a locus. Colonizer populations of the Iberian Peninsula are monomorphic and show only the most common haplotype from the original populations. Under the infinite island model and assuming that populations are in equilibrium, fixation indices indicate enough gene flow to explain why the populations are not structured. Yet, the possibility exists that populations have not reached an equilibrium after a founder event at the end of the last Pleistocene glaciation. Tajima's test suggests that directional selection and/or a recent bottleneck could explain the present mtDNA differentiation. Considering the significant population structure found for the chromosomal and some allozyme polymorphisms, the among-population uniformity for mtDNA variability argues in favor of the chromosomal and some allozyme polymorphisms being adaptive.

Introduction

The cactophilic fly *Drosophila buzzatii* is a member of the *buzzatii* species complex of the *Drosophila repleta* group (Wasserman 1992; Ruiz and Wasserman 1993). A native of the arid areas of Northwestern Argentina (Fontdevila 1989), this species is widely distributed in South America, ranging from 35° South latitude in Argentina, to extensive areas of Bolivia, Paraguay, and Brazil (Wasserman 1992). During the last 200 years, *D. buzzatii* has successfully colonized the Old World (Fontdevila 1989, 1991) and Australia (Barker 1982), following the cacti of the *Opuntia* genus, its main natural host plants.

Hypotheses concerning the deterministic (adaptive) and/or the historical (random) nature of the genetic diversity observed in both the original range of distribution and the colonizing areas must be founded on comparative studies using several genetic markers that experience selection with different intensities. These studies may also help to unveil the phylogenetic relationships between original and colonized populations. In *Drosophila,* mitochondrial DNA (mtDNA) has shown to be a powerful genetic marker to study both colonization (Latorre, Moya, and Ayala 1986; Hale and Singh 1987; Rozas et al. 1990) and population structure (DeSalle et al. [1987] for *D. mercatorum*; Baba-Aissa et al. [1988], Nigro [1988], Rand, Dorfsman, and Kann [1994], Ballard and Kreitman [1994], and Hale and Singh [1987, 1991] for *D. simulans* and *D. melanogaster*; Afonso et al. [1990] and Latorre et al. [1992] for *D. subobscura*; Halliburton and Barker [1993] for *D. buzzatii*). Because of its clonal uniparental inheritance via female parent, the effective population size (N_e) for mitochondrial genes is about one fourth of that for nuclear autosomal genes. This reduced N_e diminishes the gene flow effect compared to nuclear genes subjected to similar mutation rates and selection pressures. Consequently, population structure due to stochastic events (e.g., bottlenecks, founder events, etc.) can be detected more efficiently studying mitochondrial variation.

In the present report we performed an extensive restriction analysis of the mtDNA polymorphism from original and colonized populations of *D. buzzatii* in an attempt to unveil its history of invasion and colonization in original and new areas. We want to answer the following questions: (1) How much mtDNA genetic differentiation exists among original and colonizing populations? (2) Using the comparative approach, how much of the geographic structure unveiled by the chromosomal and allozyme studies is adaptive and how much is due to historical events? (3) Is it possible to decide which original population(s) are good candidates as founder populations in the Old World colonization? The present results give partial answers to the first two questions but are uninformative with respect to the third one.

Key words: mtDNA polymorphism, population structure, colonization, founder events, *Drosophila buzzatii*.

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Materials and Methods

Isofemale Lines of *D. buzzatii*

Two hundred and eighty-three *D. buzzatii* isofemale lines (i.e., progenies of single gravid females collected

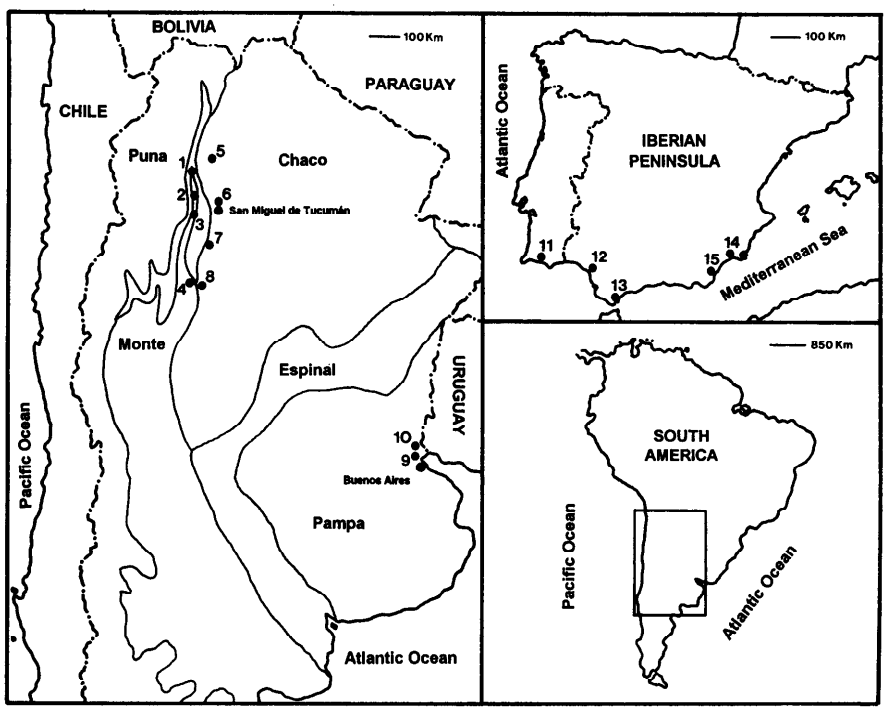

FIG. 1.—Geographic location of the populations analyzed. From Argentina, 1: Cachi; 2: Cafayate; 3: Quilmes; 4: Mazán; 5: Güemes; 6: Vipos; 7: Palo Labrado; 8: Chumbicha; 9: Arroyo Escobar; and 10: Otamendi. The populations are located in different phytogeographic regions. 1–4 in Monte, 5–8 in Chaco, and 9–10 in Pampa. From the Iberian Peninsula, 11: Albufeira; 12: Sanlúcar de Barrameda; 13: La Línea de la Concepción; 14: Mazarrón; 15: Carboneras.

in the wild) from 10 and 5 Argentinian and Iberian Peninsula populations, respectively, were analyzed. The geographic location of the New World populations, which include a major part of the actual distribution area of *D. buzzatii* in South America, comprises diverse phytogeographic regions (fig. 1). A detailed description of the localities can be found in Hasson, Naveira, and Fontdevila (1992) and Hasson et al. (1995). Figure 1 also shows the geographic location of the Iberian Peninsula populations. These populations occupy the Southern part of the peninsula where most of *Opuntia* plantations are located. Their description is as follows: Mazarrón (MAR) is a small plantation of *O. ficus-indica* surrounding an abandoned farm house, 25 km from Mazarrón on the road to Totana. Carboneras (CAR) is a large semiabandoned plantation of *O. ficus-indica* that has been described in Ruiz et al. (1986). La Linea (LIN) is a fence of several plants of *Opuntia,* possibly *O. maxima,* separating plots of cattle pastures. This locality is located close to the coast of the strait of Gibraltar, a region of high humidity, 10 km from La Linea de la Concepción. Sanlúcar (SAN) population is a thick row of plants belonging to two species, possibly *O. maxima* and *O. dillenii,* 3 km from Sanlúcar de Barrameda along the road from Chipiona to Trebujena (CN 441). Albufeira (ALB) is a hedgerow of *O. ficus-indica* plants separating cultivated fields, located in a local road 200 m from Branqueira, close to E125 road from Faro to Portimao, 7 km from Guia. This locality is in Portugal, whereas the other four are in Spain.

Extraction and Restriction Endonuclease Digestion of mtDNA

An enriched fraction of mtDNA was extracted from 15 young flies of each isofemale line by the method described by Latorre, Moya, and Ayala (1986). This preparation was sufficient for digestion with several restriction endonucleases. Fifteen restriction endonucleases were used in the present study. Two of them (*Hae*III and *Msp*I) recognize 4-bp sequences. Other enzymes (*Bcl*I, *Cla*I, *Eco*RI, *Eco*RV, *Hind*III, *Pst*I, *Pvu*II, *Sac*I, *Sca*I, *Xba*I, and *Xho*I) recognize 6-bp sequences. For statistical computation, the enzymes *Asp* 700I and *Bst*EII, which have undetermined nucleotides in their recognition sequences, were considered as 6-bp recognition enzymes as well.

Mitochondrial DNA digestion fragments were separated in 0.8%–1.2% agarose by gel electrophoresis. The fragment size was determined by using λ DNA digested with *Hind*III and lambda DNA double digested with *Hind*III and *Eco*RI as molecular weight markers. Gels were stained with ethidium bromide (0.1 mg/ml) and observed under UV light.

Bands smaller than 0.3 kb were detected by Southern blot (Southern 1975), using as probes the four *Eco*RI fragments covering the total *D. obscura* mitochondrial genome (Barrio et al. 1992), labeled by the digoxigenin-dUTP method and detected according to the manufacturer's instructions (Boehringer Mannheim).

Restriction maps for all 15 enzymes were obtained by single and double digestions of mtDNAs. The correspondence between the restriction and the gene maps was determined by comparing the conserved restriction sites shared by different *Drosophila* species (Clary and Wolstenholme 1985; Solignac, Monnerot, and Mounolou 1986; Barrio et al. 1992) and *D. buzzatii,* verified by filter hybridization with the *D. obscura* digoxigenin-labeled probes.

Number of Nucleotide Substitutions and Genetic Structure of the Populations

We used Nei's (1987) maximum likelihood method to estimate the average number of substitutions per nucleotide site between haplotypes (d_{xy}). The mtDNA differentiation both within and between populations (V_w and V_b), as well as the degree of population subdivision (N_{st}) were estimated by using equations (3), (15), and (36), respectively, from Lynch and Crease (1990). Genetic structure has also been analyzed considering restriction sites as single loci and to estimate the degree of population subdivision (G_{st}) we used the procedure developed by Takahata and Palumbi (1985). The neutral hypothesis was tested following Tajima (1989).

Results

The length of the *D. buzzatii* mtDNA was estimated as 16.0 kb with all 15 endonucleases employed. We have not detected any length polymorphism within or between populations. Additionally, all the isofemale lines analyzed proved to be homoplasmic. The 15 endonucleases yielded a total of 61 restriction sites, 20 (33%) of them were polymorphic. The restriction maps for each enzyme are shown in figure 2. Four endonucleases (*Bcl*I, *Sac*I, *Xba*I, and *Xho*I) yielded the same pattern for all the isofemale lines analyzed, while the other 11 were polymorphic. The combination of the single patterns obtained with each restriction enzyme yielded a total of 26 composite patterns or haplotypes (table 1). Figure 3 shows the network connecting all the haplotypes, which minimizes the total number of restriction site changes. As can be observed, haplotype I occupies a central position. That it is ancestral is also corroborated by its abundance and presence in all original populations (overall frequency of 61.0%). Table 2 shows the distribution of haplotypes according to the different populations sampled. All colonized populations show only haplotype I. This uniformity is most probably the result

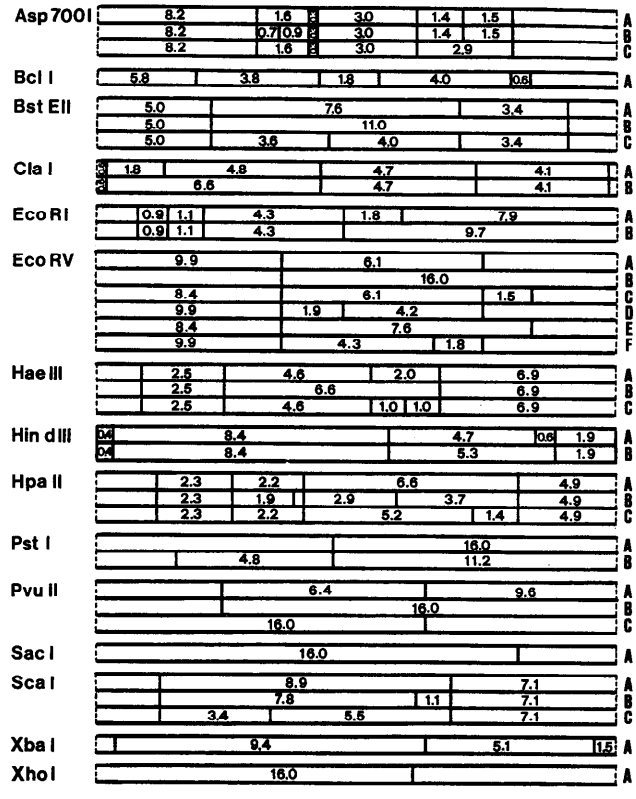

FIG. 2.—Mitochondrial DNA restriction maps of *D. buzzatii* from the 15 endonucleases employed in this study. Numbers indicate length of fragments in kilobases.

of the founder event in the colonization, but a process of random lineage extinction cannot be excluded in a secondary expansion. Haplotype II is also present in all original populations with an overall frequency of 18%, except in Mazán where the sample size is very small. Haplotypes III, IV, X, and XII were found in at least two populations. The remaining 20 haplotypes are endemic, i.e., they are present in a single population and each of them is represented by a single isofemale line. The single isofemale line with haplotype VI found in Arroyo Escobar corresponds to a previous sampling carried out 2 years before the present collection.

The number of substitutions per nucleotide site between pairs of haplotypes (d_{xy}) have been computed (see Materials and Methods). The d_{xy} values for *D. buzzatii* are similar to other *Drosophila* species (DeSalle, Giddings, and Kaneshiro 1986; DeSalle, Giddings, and Templeton 1986; Solignac, Monnerot, and Mounolou 1986; Chang, Wang, and Ayala 1989; Hale and Singh 1991; Tamura, Aotsuka, and Kitagawa 1991; Latorre et al. 1992), ranging from 0.0017 to 0.0145. It is worth noticing that the highest d_{xy} values were observed among endemic haplotypes. The whole matrix of distances is available upon request to the authors.

Table 1
Patterns Obtained with the 15 Restriction Endonucleases, which Characterizes the 26 Haplotypes of mtDNA of *D. buzzatii*

Haplotype	Restriction Endonuclease														
	*Asp*I	*Bcl*I	*Bst*EII	*Cla*I	*Eco*RI	*Eco*RV	*Hae*III	*Hind*III	*Hpa*II	*Pst*I	*Pvu*II	*Sac*I	*Sca*I	*Xba*I	*Xho*I
I	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
II	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A
III	A	A	A	A	A	C	A	A	A	A	A	A	A	A	A
IV	A	A	A	A	A	D	A	A	A	A	A	A	A	A	A
V	A	A	A	A	A	E	A	A	B	A	A	A	A	A	A
VI	A	A	A	A	A	B	A	A	A	A	A	A	B	A	A
VII	A	A	A	A	A	D	A	A	A	A	A	A	C	A	A
VIII ...	B	A	A	A	A	C	A	A	B	A	A	A	A	A	A
IX	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A
X	A	A	A	A	A	A	A	A	C	A	A	A	A	A	A
XI	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A
XII	A	A	A	A	A	C	A	A	B	A	A	A	A	A	A
XIII ...	A	A	A	A	A	D	A	A	A	A	B	A	A	A	A
XIV ...	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A
XV	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A
XVI ...	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
XVII ...	A	A	A	B	A	B	A	A	A	A	A	A	A	A	A
XVIII ..	C	A	A	A	A	B	A	A	A	A	A	A	A	A	A
XIX ...	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A
XX	A	A	A	A	A	F	A	A	A	A	A	A	A	A	A
XXI ...	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A
XXII ...	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A
XXIII ..	A	A	A	A	A	B	C	A	A	A	A	A	A	A	A
XXIV ..	A	A	C	A	A	B	A	A	A	B	A	A	A	A	A
XXV ...	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A
XXVI ..	A	A	A	A	A	A	A	A	A	A	C	A	A	A	A

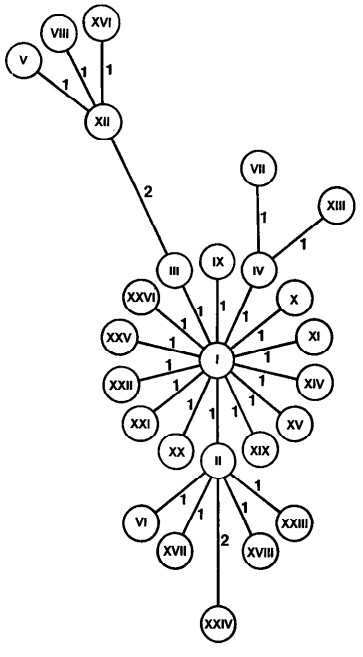

FIG. 3.—Network of the 26 mtDNA haplotypes of *D. buzzatii*. The haplotypes are connected in a way that minimizes the total number of restriction site changes. (See text for explanation.)

Table 3 shows the mitochondrial DNA differentiation within and between populations following Lynch and Crease (1990). Most of the observed variation is concentrated within populations. The total amount of mtDNA polymorphism (i.e., nucleotide diversity) may be estimated by the average number (V_w) of substitutions per nucleotide site for random pairs of haplotypes from the same population plus the average number (V_b) between populations. For original populations only (data not shown), V_w and V_b values are 0.00148 ± 0.00106 and 0.00003 ± 0.00074, respectively, and the N_{st} statistic that measures the fraction of nucleotide variation that can be accounted for by V_b (Lynch and Crease 1990) is 0.023, a value statistically not different from zero. When all populations are considered, both values are 0.00119 ± 0.00078 and 0.00008 ± 0.00065 and the N_{st} statistic is 0.060 ± 0.471, which is also not significantly different from zero ($P < 0.05$). Similar analyses have been carried out by combining the haplotype distribution corresponding to Argentinian populations belonging to the same phytogeographic region (Cabrera 1976). In this way Cachi, Cafayate, and Quilmes belong to the phytogeographic region named Monte; Güemes, Vipos, Palo Labrado, and Chumbicha belong to the region named

Table 2
Number of Isofemale Lines for the 26 Haplotypes in the Populations of *D. buzzatii* Analyzed

Haplotype	Population																
	Argentina										Iberian Peninsula						
	CAC	CAF	QUI	MAZ	GUE	VIP	PAL	CHU	ARR	OTA	ALB	SAN	LIN	CAR	MAR	Total	%
I	11	17	9	3	15	17	18	4	11	14	4	23	20	29	12	207	73.1
II	5	1	2	0	4	5	3	4	6	6	0	0	0	0	0	36	12.7
III	1	0	1	0	0	2	0	0	0	1	0	0	0	0	0	5	1.8
IV	1	0	0	0	0	1	2	0	1	0	0	0	0	0	0	5	1.8
V	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0.4
VI	0	0	0	0	0	0	0	0	(1)	0	0	0	0	0	0	1	0.4
VII	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0.4
VIII ...	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0.4
IX	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0.4
X	1	0	0	0	0	2	2	1	0	1	0	0	0	0	0	7	2.5
XI	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0.4
XII	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	3	1.1
XIII ...	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0.4
XIV ...	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0.4
XV	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0.4
XVI ...	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.4
XVII ...	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.4
XVIII ..	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0.4
XIX ...	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0.4
XX	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.4
XXI ...	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0.4
XXII ...	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0.4
XXIII ..	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0.4
XXIV ..	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0.4
XXV ...	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0.4
XXVI ..	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0.4
Total ...	21	19	13	3	22	30	28	13	20	26	4	23	20	29	12	283	

NOTE.—The abbreviations for the populations are as follows: CAC (Cachi), CAF (Cafayate), QUI (Quilmes), MAZ (Mazán), GUE (Güemes), VIP (Vipos), PAL (Palo Labrado), CHU (Chumbicha), ARR (Arroyo Escobar) OTA (Otamendi), ALB (Albufeira), SAN (Sanlúcar de Barrameda), LIN (La Línea de la Concepción), MAR (Mazarrón), and CAR (Carboneras). The isofemale line with haplotype VI (which is in parentheses) was collected in Arroyo Escobar several years before the remaining isofemale lines.

Chaco; and, finally Arroyo Escobar and Otamendi are typical Pampa populations (see fig. 1). Mazán has been excluded from this analysis because of the low number of individuals sampled. The average within- and between-region variations are 0.00190 ± 0.00105 and 0.00002 ± 0.00080, respectively, and the corresponding fraction of nucleotide variation that is due to between-region variation is 0.010 ± 0.411 (see table 4).

Similar results have been obtained when considering each restriction site as a locus (Takahata and Palumbi, 1985). Table 5 is a summary of the within- and between-region identities that have been used to estimate the G_{st} in the three Argentinian phytogeographic regions. Following this method, the average value of population subdivision, G_{st}, is 0.176, one order of magnitude higher than the N_{st} value obtained when nucleotide differences between haplotypes are considered. Despite this difference (see Lynch and Crease [1990] for a proper explanation), both G_{st} and N_{st} indicate sufficient mtDNA gene flow to prevent significant population structure. If we assume that the populations are in equilibrium and that they are following an island model (Wright 1969) then the average net number of immigrants per generation, N_m, can be indirectly estimated from $Nm = (1/2)(1/N_{st} - 1)$ or $(1/2)(1/G_{st} - 1)$, where N is the effective number of females and m the average fraction of a population that is replaced by immigrants in any generation (Slatkin 1989). In both cases the Nm is higher than 1, the reference value to consider that the phytogeographical regions are not isolated (Wright 1969).

In order to test for departures from neutral theory Tajima's test (Tajima 1989) has been used. The rationale of the test is that in a panmictic population under the neutral mutation model no difference would be expected between the estimates of heterozygosity based on the number of segregating sites (Watterson 1975) and that based on the average number of nucleotide differences (Tajima 1983). D values (equation 38, Tajima 1989) for original populations (except Mazán that is monomor-

Table 3
Mitochondrial DNA Differentiation ($\times 10^5$)

Population	CAC	CAF	QUI	MAZ	GUE	VIP	PAL	CHU	ARR	OTA	ALB	SAN	LIN	CAR	MAR
CAC ..	235	6	−3	14	−3	−6	0	−10	−5	−6	14	14	14	14	14
	(139)	(52)	(13)	(86)	(0)	(0)	(0)	(8)	(0)	(0)	(86)	(86)	(86)	(86)	(86)
CAF ..		58	−1	0	0	4	3	21	12	10	0	0	0	0	0
		(61)	(33)	(23)	(38)	(32)	(20)	(120)	(70)	(45)	(23)	(23)	(23)	(23)	(23)
QUI ...			107	2	−3	−3	−0	8	3	3	2	2	2	2	2
			(98)	(51)	(19)	(0)	(0)	(78)	(44)	(0)	(51)	(51)	(51)	(51)	(51)
MAZ ..				0	5	9	5	31	20	19	0	0	0	0	0
				(0)	(57)	(70)	(59)	(166)	(92)	(81)	(0)	(0)	(0)	(0)	(0)
GUE ..					124	−1	2	9	−1	1	5	5	5	5	5
					(101)	(0)	(0)	(67)	(41)	(0)	(57)	(57)	(57)	(57)	(57)
VIP ...						225	−5	−2	2	0	9	9	9	9	9
						(133)	(0)	(27)	(0)	(0)	(70)	(70)	(70)	(70)	(70)
PAL ..							209	6	11	9	5	5	5	5	5
							(132)	(63)	(0)	(0)	(59)	(59)	(59)	(59)	(59)
CHU ..								414	0	−6	31	31	31	31	31
								(190)	(35)	(8)	(166)	(166)	(166)	(166)	(166)
ARR ..									161	−3	20	20	20	20	20
									(116)	(0)	(92)	(92)	(92)	(92)	(92)
OTA ..										246	19	19	19	19	19
										(138)	(81)	(81)	(81)	(81)	(81)
ALG ..											0	0	0	0	0
											(0)	(0)	(0)	(0)	(0)
SAN ..												0	0	0	0
												(0)	(0)	(0)	(0)
LIN ...													0	0	0
													(9)	(0)	(0)
CAR ..														0	0
														(0)	(0)
MAR ..															0

NOTE.—Mitochondrial DNA differentiation ($\times 10^5$), with standard errors in parentheses, within and between populations of *D. buzzatii*. Values on the main diagonal are within-population differentiation, V_w, values above the diagonal are between-populations differentation, V_b. The abbreviations for populations are as in table 2.

phic) are negative and range between −1.233 and −1.719. All these values are statistically not different from zero ($P > 0.05$). Considering all the isofemale lines of the original populations as belonging to a single panmictic population, the D value obtained is −1.901, which is a negative value significantly different from 0 at the 0.05 level.

Discussion

The most striking result of the present study is that the South American *D. buzzatii* populations seem to be homogeneous genetically according to mtDNA polymorphism. This result cannot be accounted for by error sampling because the number of isofemale lines analyzed was high enough. This homogeneity is not the rule in most studies with other species (Moritz, Dowling, and Brown 1987). An interesting difference is that here the majority of nucleotide diversity is allocated to intrapopulation or intraregion variability in spite of the large ecological differences among the studied areas. This genetic homogeneity could be related to two issues: the predominance of haplotype I in all populations and the

Table 4
Mitochondrial DNA Differentiation

Region	Monte	Chaco	Pampa
Monte	0.00141 ± 0.00097	0.00000 ± 0.00000	0.00002 ± 0.00070
Chaco		0.00223 ± 0.00126	0.00004 ± 0.00077
Pampa			0.00207 ± 0.00123

NOTE.—Mitochondrial DNA differentiation, with standard errors in parentheses, within and between the three Argentinian biogeographic regions (Monte, Chaco, and Pampa) where populations of *D. buzzatii* have been sampled. Values on the main diagonal are within-region differentiation, V_w; values above the diagonal are between-region differentiation, V_b.

Table 5
Within- (on the Diagonal) and Between-region (Above the Main Diagonal) Identity Probabilities for the Three Argentinian Biogeographic Populations of *D. buzzatii*

	Monte	Chaco	Pampa
Monte	0.881	0.835 (0.100)	0.818 (0.166)
Chaco		0.849	0.804 (0.146)
Pampa			0.858

NOTE.—Between parentheses are indicated the G_{st} values for pairs of biogeographic regions.

extremely low frequency of the endemic haplotypes (table 2). The unique presence of haplotype I in the Iberian Peninsula is compatible with a recent colonization but does not give information on the geographical origin of the founder population. These results are similar to those reported by Halliburton and Barker (1993) in the colonization of Australia by *D. buzzatii.*

Lack of mtDNA differentiation among original populations suggests widespread gene flow. This agrees with similar results obtained with allozymes, where under the infinite-island model the estimated N_m values are greater than 1 for most allozyme loci in original and colonizing populations (Fontdevila 1991). Direct estimates of dispersal using mark–recapture methods indicate high levels of vagility of *D. buzzatii* (Fontdevila et al., unpublished data; Barker, East, and Christiansen 1989) and other cactophilic species (Johnston and Heed 1976) and corroborates that gene flow may be a consequence of this high dispersal.

The negative value of Tajima's *D* estimated for the total data set is compatible with certain kinds of selection, such as recent directional selection, and also with population bottlenecks. Thus, the predominance of haplotypes I and II could be interpreted as the consequence of a selective sweep and also as the effect of random drift due to periodical bottlenecks. Fos et al. (1990) demonstrated the important effect of random drift in experimental populations of *D. subobscura.* They observe that, in spite of positive selection favoring one of the competing haplotypes under a uniform nuclear background, random drift influences the final destination of competing haplotypes. The lack of statistical significance of Tajima's *D* values for individual populations of *D. buzzatii* could be explained if they have not reached equilibrium due to these periodic bottlenecks, as it was suggested in a seasonal study in *D. subobscura,* in which also a high predominance of two haplotypes exists (González et al. 1994).

The alternative (but not mutually exclusive) explanation to periodic bottlenecks coupled with gene flow, postulates a recent founder event followed by an explosive invasion of NW Argentina. The cycles of glaciations in late Pleistocene have changed the distribution of arid vs. rainforest areas in tropical South America. The last episode occurred between 13,000 and 18,000 years ago (Ab'Saber 1977) when the global warming led to an increase of rain precipitation in the tropics that allowed the expansion of the present Amazonian rainforest into extensive arid and semiarid zones that occupied most of the tropics during the last glaciation. This led to important changes in the distribution of the vegetation and particularly to the fragmentation of the cactus habitat (Baimai, Sene, and Pereira 1983), in which *D. buzzatii* breeds and feeds. This fragmentation may have produced bottlenecks in the cactophilic *Drosophila* populations, in particular one of these small populations in which haplotype I was dominant might have been responsible of the recolonization of the present distribution of *D. buzzatii* in the NW Argentina.

In original populations, the relative insensitiveness of mtDNA to gene flow does not unveil any population structure that could be responsible for the geographical structure observed for chromosomal and allozyme polymorphisms. Extensive studies on the chromosomal polymorphism in the original South American populations (Fontdevila et al. 1982; Hasson et al. 1995) show that rearrangement frequencies vary clinally with altitude, latitude, and longitude. Latitudinal clines exist also in the colonizing Australian populations (Knibb and Barker 1988) and in the Old World populations (Ruiz 1982). On the other hand, the hierarchical analysis of fixation indices unveiled a significant macrogeographical regional pattern in the original populations but not in the colonized populations (Fontdevila 1991; Hasson et al. 1995). All this information suggested that the simplest explanation for chromosomal differentiation in South America would invoke ecological regional gradients, whereas founder events and latitudinal gradients would be mostly responsible for differentiation in the Old World and Australia. Studies with allozyme markers are much less extensive in the original area where only five populations have been studied. Fixation indices both in original and colonized areas are significant for many allozyme loci and there is spatial heterogeneity across loci (Fontdevila 1991). Although fixation due to founder events has produced a skewed F_{st} distribution increasing the frequency of low values, Old World colonization did not change the overall average F_{st} values for variable loci but it did in Australian colonization, a difference that can be explained by the slow comparative population expansion in the former colonization. These studies indicate that allozyme differentiation is strongly influenced by historical events, but also that natural selection is operating in some allozyme loci.

Table 6 shows a summary of population differentiation for chromosome rearrangements, allozymes, and

Table 6
Measures of Population Differentiation for Several Genetic Markers of *D. buzzatii*

	Original Populations (New World)		Colonizer Populations (Old World)
	F_{st}	F_{st} Ratio[a]	
Chromosome rearrangements[b] (F_{st})			
Chromosome 2	0.177	0.994	0.089
Chromosome 4	0.655	0.195	0.080
Allozymes[c] (F_{st})			
Xdh	0.059	2.983	0.000
Ao	0.029	6.069	0.117
Est-1	0.075	2.347	0.080
Est-2	0.216	0.815	0.198
6Pgdh	0.575	0.306	0.534
Pep-2	0.063	2.794	0.051
Lap-1	0.067	2.627	0/0
Fum	0.029	6.069	0/0
Aph	0.027	6.519	0/0
Acph	0.042	4.190	0/0
mtDNA (G_{st})	0.176		0/0

[a] F_{st} ratio: means G_{st}(mtDNA)/F_{st}(nuclear DNA).
[b] Data from Hasson et al. (1995).
[c] Data from Sánchez (1986).

mtDNA haplotypes in New (original) and Old World (colonizer) populations. To make comparisons more reliable, we have used the same populations or only populations belonging to the same geographical area. Because the quantification of significant differences among F_{st} has not been fully developed, comparisons have been performed simply by doing an F-test of the ratio of population variance of gene frequencies for two markers, using frequencies of one allele versus the rest in order to avoid problems of different number of alleles in F_{st} comparisons (McDonald 1994). It is apparent from a visual inspection of table 6 that F_{st} comparisons between mtDNA haplotypes and chromosome rearrangements do not agree with the neutral model for both markers. Under this model, mtDNA F_{st}'s are expected to amount to four times larger than those of autosomal markers, because the effective population sizes of mitochondrial and autosomal genes differ by a factor of four. Using the same 10 populations for both markers, differences in genetic variance between second chromosome and mtDNA are statistically not significant ($F_{9,9} = 1.012$; $P = 0.49$) and differentiation for chromosome 4 is significantly higher than for mtDNA ($F_{9,9} = 3.317$; $P = 0.044$). Comparisons among allozyme and mitochondrial markers are less reliable because some localities are not common to both studies and also because the low number of populations in allozyme studies (five) gives a low number of degrees of freedom and lowers the potency of the F-test. Thus, only 1 of the 10 possible comparisons is significantly different from 1 (Aph: $F_{9,4} = 6.127$; $P = 0.048$), yet F_{st} ratios have a wide range of variation. Most of them are greater than 1, ranging from 6.519 to 2.347, including the expected value of 4. Jointly, these data support the view that chromosomal markers are selective and suggest that most of the allozymes behave as neutral markers, except perhaps in those two cases (Est-2 and 6Pgdh) in which the F_{st} ratio is lower than 1. This is not the first case in which population differentiation of mtDNA is largely independent of nuclear gene variation (e.g., Hale and Singh 1991; Karl and Avise 1992; and references therein), but the results reported here argue against historical events and favor the diversifying selective hypothesis to explain the present population diversity in some allozyme loci and the geographical variation of chromosomal rearrangements.

As for the mtDNA variation, a founder event in the recent past followed by periodic population bottlenecks coupled with migration are mechanisms that may explain the geographic uniformity of mtDNA variation, but the possibility exists that some kind of selective sweep in favor of haplotypes I and II and/or selection against rare haplotypes are other explanatory mechanisms. Only future studies of nucleotide diversity may help to solve this uncertainty.

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