

Population Dynamics and Evolution of B Chromosomes

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Spatio-temporal dynamics of a neutralized B chromosome in the grasshopper *Eyprepocnemis plorans*

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Abstract. Spatial and temporal patterns of frequency variation for a neutralized B chromosome in the grasshopper *Eyprepocnemis plorans* were analyzed along six transects in the east of Spain to explore possible factors affecting the population dynamics of this polymorphism. Three parameters were employed to quantify B frequency: prevalence, load and mean frequency. Of them, load seemed to be the less sensitive parameter, probably due to its small range of variation. Prevalence, however, shows ample variation, but the mean frequency of B chromosomes per individual is the best parameter to characterize B frequency. Only river transects revealed significant differences among populations, and the use of two geographic explicit approaches (Mantel test and distograms) revealed significant

isolation by distance (IBD), especially at the Segura River mouth, presumably due to low gene flow and drift. No temporal trend was found in the Segura River transects, which is consistent with the slow changes in B frequency expected during the random walk for neutralized B chromosomes. But these transects showed a clear spatial pattern, with B₁ showing lower frequency in the upper course of this river. The present results provide the first empirical evidence of IBD in the evolution of a neutralized B chromosome, and support the notion that B dynamics at this evolutionary stage is best explained by a meta-population approach.

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The grasshopper *Eyprepocnemis plorans* subsp. *plorans* shows a very widespread polymorphism for B chromosomes ranging from the west of the Iberian Peninsula (e.g. Huelva, Spain) to the Caucasus (e.g. Dagestan) (for review, see Camacho et al., 2003). Only in the Iberian Peninsula, more than 50 B variants have been found differing in size, morphology and C-banding (see Camacho et al., 2003), and more variants are continuously being found (Bakkali and Camacho, 2004) suggesting that this species Bs are very dynamic.

The analysis of population dynamics of B chromosomes in the grasshopper *E. plorans* has illuminated the long-term evolution of these parasitic elements (Camacho et al., 1997). The life

cycle of a parasitic B chromosome begins with a rapid B invasion by virtue of drive leading it to a high frequency. The A chromosome response may imply the suppression of B drive which is thus neutralized to evolve through a long random walk towards extinction due to selection against individuals with many Bs. But the B may mutate and recuperate drive thus restarting the cycle and greatly prolonging the life of the B chromosome polymorphism. This has been named regeneration and has been directly witnessed in Torrox (Málaga, Spain) by Zurita and colleagues (1998).

The most widespread B variant of *E. plorans* in the Iberian Peninsula, named B₁, might be the oldest B chromosome in this region (Henriques-Gil et al., 1984). Its size is about half that of the X chromosome and appears positively heteropycnotic in meiotic prophase, although it shows two dark C-bands in the proximal third. These dark C-bands contain a 180-bp tandem-repeat DNA (satDNA) and the remaining long arm (the B also has a very short arm which is not always conspicuous) is made of ribosomal DNA (rDNA) (López-León et al., 1994). B₁ has been found over the whole southeastern coast, from Tarragona to Huelva, with only two exceptions: (1) the Granada and eastern Málaga coasts, where it has been substituted by B₂, a small-

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Table 1. Transects analyzed with indication of the number of populations sampled

Area/Transect	Year	Number of populations	Individuals	Sampled individuals per population
Segura River	1993	13	239	18.4±2.5
	1996	8	188	23.5±2.4
	1998	17	576	33.9±0.9
	1999	13	373	28.7±1
	2000	18	451	25.1±0.7
	2001	17	427	25.1±0.7
Segura River mouth	1992	12	243	20.3±2
Mar Menor Lagoon	1992	16	312	19.5±2
Coast of Valencia	1995	23	421	18.3±1.2
Turia River	1995	10	222	22.2±2
Jucar River	1996	12	270	22.5±0.9

er variant bearing relatively more satDNA but less rDNA, and (2) Fuengirola, where it has been replaced by B₅, another variant carrying more satDNA (Henriques-Gil and Arana, 1990). B₁'s geographical distribution also includes Morocco (Bakkali et al., 1999) and the Mallorca Island (Riera et al., 2004). B₁, B₂ and B₅ showed a Mendelian transmission rate (López-León et al., 1992). The specimens analysed for B₁ transmission came from the east of the Iberian Peninsula. In Morocco, however, B₁ showed a Mendelian transmission rate in two populations but significant accumulation in a third one, precisely the southern one (Mechra) (Bakkali et al., 2002). The only known region lacking B chromosomes in the Iberian Peninsula is an inland region at the head of the Segura River basin, which could be remnants of ancient populations never reached by the Bs. The closest B-carrying populations, downstream of the Cenajo reservoir in the Segura River, harboured B₁ (Cabrero et al., 1997).

Spatial patterns may be used to study a wide range of genetics processes, from migration to selection to population contraction and range expansion (Epperson, 2003). Analysis of spatial patterns of genetic variation can be used to study gene flow and natural selection (Brashaw, 1984; Slatkin, 1987) and to evaluate the relative historical influence of gene flow and drift on regional population structure (Hutchinson and Templeton, 1999). The most common spatial pattern is isolation by distance (Epperson, 2003), produced basically by a reduction in the dispersal of the individuals. Under isolation by distance (IBD), genetic differences between populations grow proportionally to the physical separation of these populations. IBD should affect in similar form all neutral genes, but selection could affect the frequency of some genes according to specific patterns (Heywood, 1991). In addition, unless migration is irrelevant, spatial patterns are highly dependent on the status of underlying spatial-temporal processes, except in cases of strong clinal selection (Epperson, 2003).

On the basis of the model described at the beginning of this section, the B₁ chromosome might be considered a neutralized variant since it does not drive (López-León et al., 1992), with the above-mentioned exception of Mechra in Morocco. In this paper, we analyze spatial and temporal patterns of B₁ frequency variation in six transects in the east of Spain to explore possible factors affecting the population dynamics of this neutralized B chromosome.

Materials and methods

A total of 3,722 adult males of the grasshopper *E. plorans* were collected from populations along six transects in the Spanish provinces of Murcia, Alicante and Valencia during 1993–2001 (see Table 1 and Fig. 1). Testes were fixed in freshly prepared 3:1 ethanol:acetic acid and stored at 4 °C. A mean of 23.4 ± 7.3 males were analyzed per population/year. For scoring the number of Bs in each individual, squash preparations of single follicles in a drop of 45 % acetic acid were made and observed under phase contrast. Individuals were classified as 0B, 1B, 2B or 3B.

Contingency χ^2 tests were done to analyze population differences in the distribution of individuals with different number of Bs in each transect. These tests were performed with the RXC program (George Carmody, University of Ottawa, Canada) by a Monte Carlo approach to calculate the statistical significance of the contingency table. Tests were conducted with 10,000 permutations.

Three frequency parameters were calculated: (1) prevalence, the proportion of individuals carrying B chromosomes; (2) load, the mean number of B chromosomes in B-carrying individuals; and (3) mean frequency of the B chromosome, calculated as the average number of B chromosomes per individual considering carriers and noncarriers.

Geographic maps were digitalized and distances between populations were calculated with the program ImageJ (<http://rsb.info.nih.gov/ij/>). A matrix of geographical distances between populations (in km) was obtained for each area sampled. For populations along the course of a river, distances were calculated following the course of the river, i.e., in a uni-dimensional space. For the other populations, distances were obtained "as the bird flies", i.e., as straight lines in a bi-dimensional space.

Three matrices of disagreement between populations (one for each genetic parameter) were obtained, where the individual values of the matrix were calculated as the absolute difference of the genetic parameter between each pair of populations.

To explore possible geographical patterns, we compared the geographical matrix with each of the three genetic matrices by means of the Mantel test. For populations in a bidimensional space, geographical distances were log-transformed, following Slatkin (1993). The significance of the Mantel test was obtained by 10,000 permutations with the program zt (Bonnet and Van de Peer, 2002).

When an association between a genetic parameter and the geographic distance was found, an additional analysis was performed. We calculated a measure of spatial autocorrelation based on genetic distances using SGS software (Degen et al., 2001). To adapt the B-chromosome frequency to data comparable to gene frequencies, we divided the mean B₁-chromosome frequency (a value referred to individuals) by two, to obtain a value referred to as haploid data. We used the Gregorius distance (Gregorius, 1978), calculated for a single locus (B chromosome) as

$$D_G(i, j) = \frac{1}{2} \sum |p_i - p_j|$$

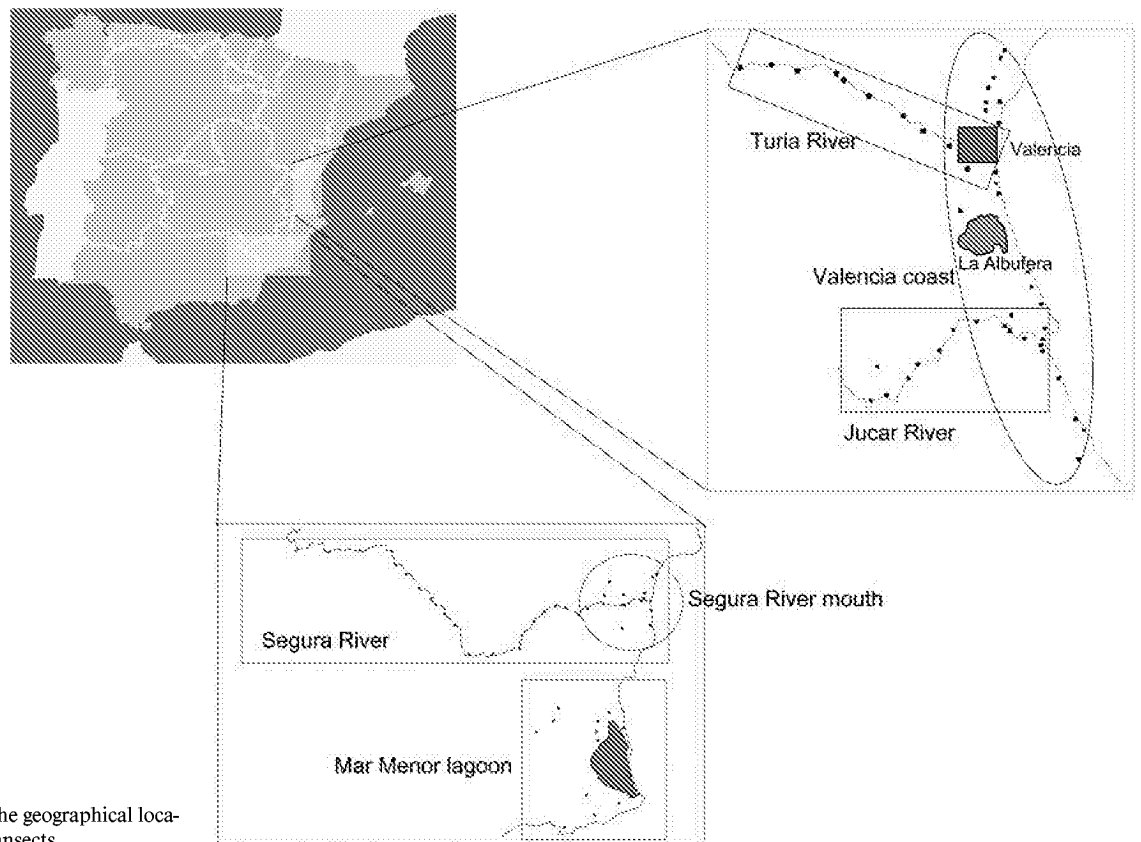


Fig. 1. Map showing the geographical location of the six analyzed transects.

where i and j are populations, and p_i and q_j the frequency of the B_1 chromosome in each population, because it has a simple meaning when adapted to B-chromosome frequencies (i.e., $D_G = 1$ implies one population with zero Bs and another one with all the individuals with two B chromosomes).

SGS calculates "genetic distograms" as representation of the average genetic distance of all pairs of populations belonging to a particular distance interval (D_{Gsq}). The mean genetic distance over all pairs of populations was used as a reference value. D_{Gsq} below the reference value implies a positive spatial structure, i.e. populations are genetically more similar than expected for a spatially random distribution (Degen, 2000). Values of D_{Gsq} over the reference value imply a negative structure, where populations are more divergent than expected for a spatially random distribution (Degen, 2000). Confidence intervals were calculated by 1,000 permutations. Several spatial distance intervals were used because the scale at which spatial structure is produced is a priori unknown.

To explore possible temporal variation in prevalence, load and mean frequency of the B_1 chromosome from the populations of the Segura River, we performed multiple regression analysis of these variables considering distance to the coast and year as independent factors. The correlation coefficient for year may be used to test for a temporal trend.

Results

The mean prevalence for the different transects was 34.05% \pm 1.24, and ranged from 0 to 73.91% (see Table 2). The load ranged from 0 (an arbitrary value to indicate the absence of Bs) to 2, with a mean value of 1.13 \pm 0.02, and the mean B frequency from 0 to 0.916, with a mean of 0.405 \pm 0.017 (Fig. 2).

Segura River populations

These populations were sampled six times during a period of nine years, in a transect of more than 145 km along the river course. However, the capture sites were not always the same, precluding an exhaustive temporal analysis.

We analyzed the null hypothesis of no differentiation among populations by using contingency χ^2 tests for each year data, i.e., using a non-explicit geographic approach. Only populations sampled the year 2000 rejected the null hypothesis ($\chi^2 = 87.13$, $P = 0.002$).

When we compared, by the Mantel test, the matrices of distances along the river with the matrices of differences in prevalence, they did not show association in 1993 ($r = -0.083$, $P = 0.321$), 1996 ($r = 0.363$, $P = 0.115$) and 1999 ($r = 0.125$, $P = 0.129$), but they did in 1998 ($r = 0.285$, $P = 0.029$), 2000 ($r = 0.224$, $P = 0.035$) and 2001 ($r = 0.314$, $P = 0.012$). The positive sign of this association implies that more distant populations have higher differences in B_1 prevalence.

Data from 1993 ($r = 0.002$, $P = 0.425$), 1996 ($r = -0.0002$, $P = 0.580$), 2000 ($r = 0.039$, $P = 0.3149$) and 2001 ($r = 0.047$, $P = 0.251$) did not show association between distance and load for the B_1 chromosome. However, a positive relationship was found in 1998 ($r = 0.388$, $P = 0.001$), and a negative one in 1999 ($r = -0.203$, $P = 0.034$).

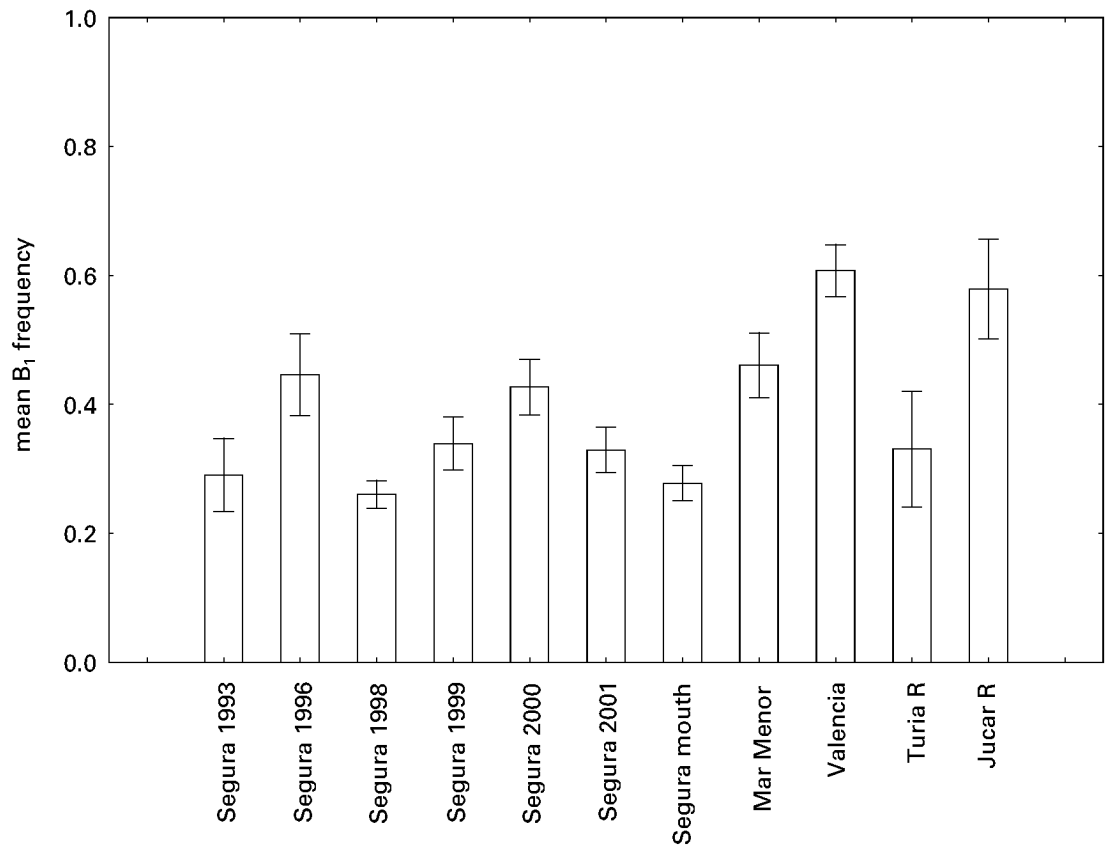


Fig. 2. Mean B₁ frequency in the sampled transects. Error bars represent ± one standard error.

Table 2. Mean ± S.E. for prevalence, load and mean B frequency for each transect analyzed. Between parentheses are the maximum and minimum values found

Area/transect	Year	Prevalence	Load	Mean B frequency
Segura River	1993	0.263±0.048 (0–0.667)	1.059±0.116 (0–2)	0.291±0.057 (0–0.833)
	1996	0.328±0.045 (0.05–0.448)	1.348±0.111 (1–2)	0.446±0.063 (0.05–0.63)
	1998	0.235±0.019 (0–0.316)	1.044±0.070 (0–1.333)	0.260±0.022 (0–0.395)
	1999	0.297±0.031 (0.067–0.467)	1.127±0.041 (1–1.417)	0.339±0.041 (0.067–0.567)
	2000	0.364±0.030 (0.042–0.579)	1.146±0.031 (1–1.5)	0.427±0.043 (0.042–0.833)
	2001	0.294±0.028 (0.053–0.478)	1.102±0.027 (1–1.273)	0.329±0.035 (0.053–0.609)
Segura River mouth	1992	0.259±0.028 (0.125–0.429)	1.094±0.047 (1–1.5)	0.278±0.027 (0.125–0.429)
Mar Menor Lagoon	1992	0.389±0.04 (0–0.667)	1.102±0.081 (0–1.417)	0.461±0.05 (0–0.792)
Valencia coast	1995	0.473±0.02 (0.25–0.6)	1.258±0.041 (1–1.6)	0.608±0.04 (0.25–0.917)
Turia River	1995	0.286±0.073 (0–0.625)	0.915±0.16 (0–1.4)	0.331±0.09 (0–0.875)
Jucar River	1996	0.469±0.057 (0.091–0.739)	1.202±0.04 (1–1.444)	0.579±0.077 (0.091–0.913)

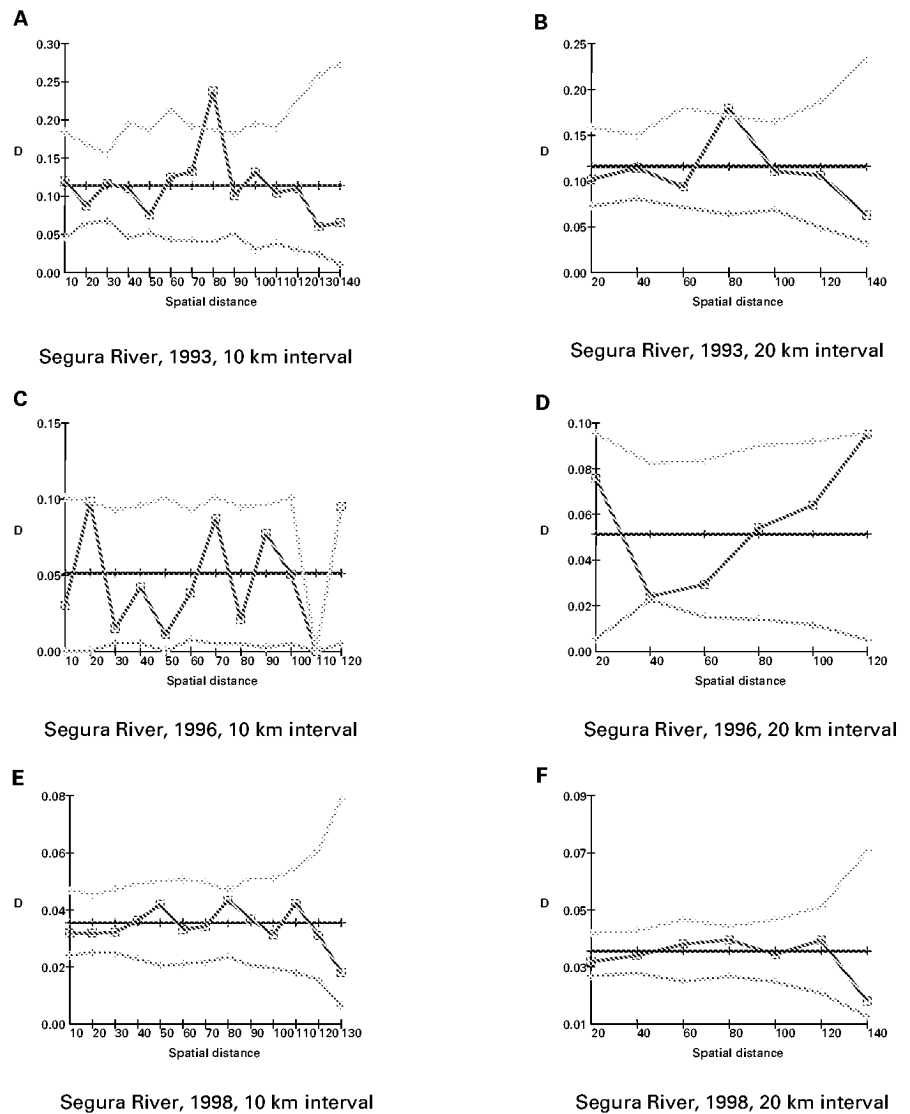


Fig. 3. Distograms for Gregorius distances at the Segura River transect for years 1993, 1996 and 1998. The horizontal line represents the overall Gregorius distance mean. Lines with squares represent the mean Gregorius distance at each geographical distance interval. Continuous lines represent 0.95 confidence intervals.

The relationship between differences in mean B_1 frequency and distance along the river was not significant in 1993 ($r = -0.053$, $P = 0.430$) and 2001 ($r = 0.171$, $P = 0.073$), but it was positive and significant in 1996 ($r = 0.526$, $P = 0.013$), 1998 ($r = 0.265$, $P = 0.036$), 1999 ($r = 0.222$, $P = 0.042$) and 2000 ($r = 0.287$, $P = 0.017$). In general, the data showed a positive relationship between distance and population dissimilarity in respect to B_1 frequency, a situation that is usual under isolation by distance.

To explore the relationship between these populations at different spatial scales, we analyzed B_1 chromosome frequency data (see Materials and methods) by means of distograms. Fig. 3 and 4 show distograms with 10- and 20-km interval classes for the Segura River populations. In most years sampled, the Gregorius distances among populations did not significantly vary with geographic separation but, in several years, especially 2000 and 2001, there was a significant increase in genetic distance (i.e., dissimilarity among pairs of populations) at scales over 80 km.

To explore possible temporal trends, we performed a multiple regression analysis to these data, considering year of sampling and distance to the coast along the river as independent factors. The analysis of the multiple regression coefficients showed that year of sampling was not associated with prevalence ($P = 0.208$), load ($P = 0.935$) and mean frequency ($P = 0.935$) of Bs in these populations. However, the distance to the coast was a factor explaining part of the variance of prevalence ($\beta = -0.282$, $P = 0.008$) and mean frequency ($\beta = -0.215$, $P = 0.047$) of B_1 , but not load ($P = 0.668$). Prevalence and mean frequency decreased with the distance to the coast (see Fig. 5).

Segura River mouth populations

These twelve populations were sampled in 1992 in an area of about 15×15 km, around the mouth of the Segura River. The contingency χ^2 test failed to show significant differences among these populations ($\chi^2 = 25.91$, $P = 0.345$).

To explore spatial patterns, we compared the matrix of log-transformed geographic distances with the matrices of B_1 dis-

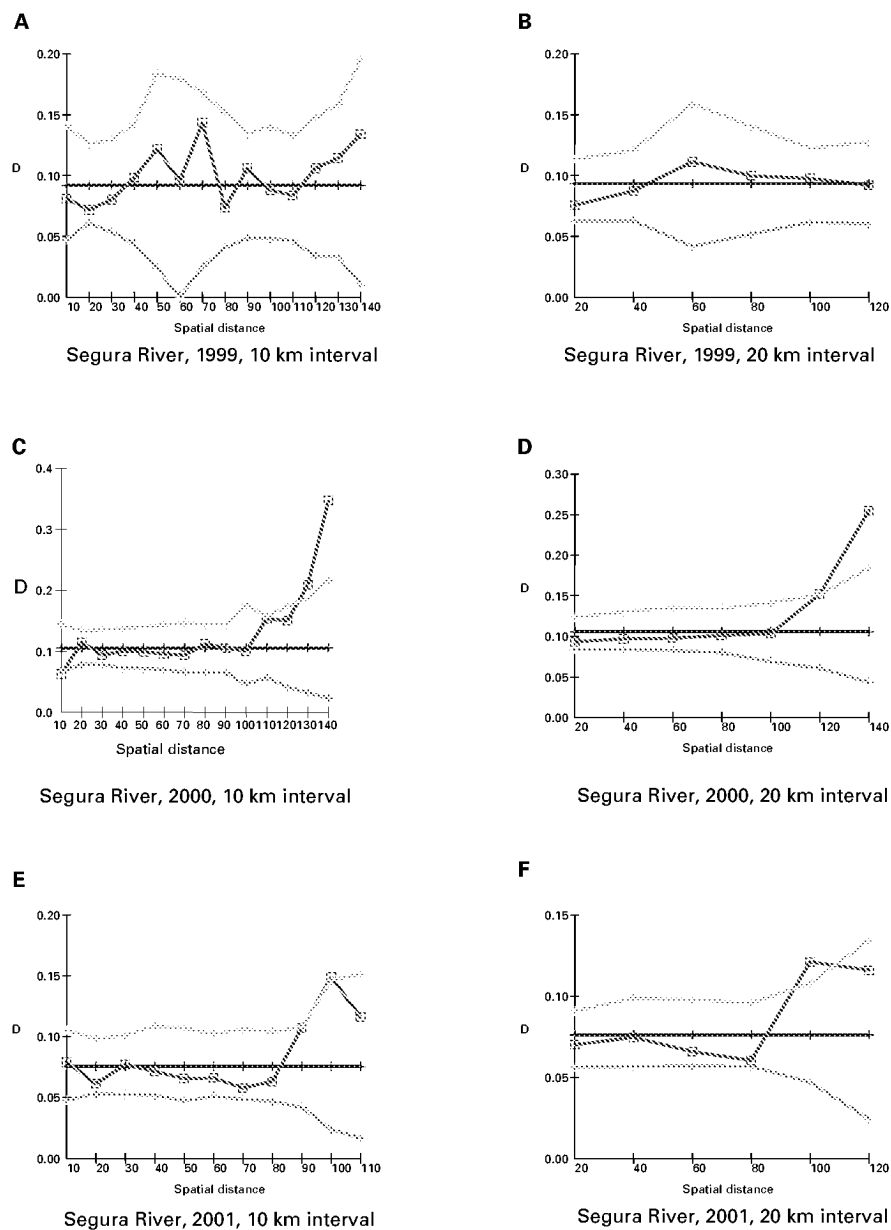


Fig. 4. Distograms for Gregorius distances at the Segura River transect for years 1999, 2000 and 2001.

similarities (prevalence, load and frequency). For prevalence and load there were no significant associations ($r = 0.108$, $P = 0.198$, and $r = -0.097$, $P = 0.342$, respectively), but there was for mean B_1 frequency ($r = 0.257$, $P = 0.037$), implying an increased difference between populations in relation to geographic distance. A similar pattern was apparent with the distogram analysis (see Fig. 6). At a spatial scale of 3 km, there was significant increased similarity (i.e. lower genetic distance than expected) at low geographical distances (4–6 km class, $P = 0.029$), and decreased similarity at higher distances (8–10 km, $P = 0.012$). Similar results were found when the distance interval was 3 km (see Fig. 6), when significant deviation at 3–6 km ($P = 0.019$) and 9–12 km ($P = 0.037$) classes. This pattern is typical of isolation by distance.

Mar Menor Lagoon populations

This transect ran around the Mar Menor Lagoon, mainly along its inland shore, and included 16 sampled populations separated between a minimum of 1.7 km up to 34.97 km. As in the previous transect, the contingency χ^2 test did not show significant differences among populations ($\chi^2 = 39.49$, $P = 0.661$). The explicit geographic analysis using Mantel tests showed no association between log-transformed geographic distances and either prevalence, load or mean frequency ($r = 0.105$, $P = 0.176$; $r = -0.107$, $P = 0.224$; $r = 0.002$, $P = 0.470$, respectively).

The spatial analysis using distograms also showed that populations were homogeneous for B frequency (see Fig. 6c) at low spatial classes, but at the maximum distance (30–35 or 30–40 km classes) they were more similar than expected by chance. The general aspect of these distograms are compatible with

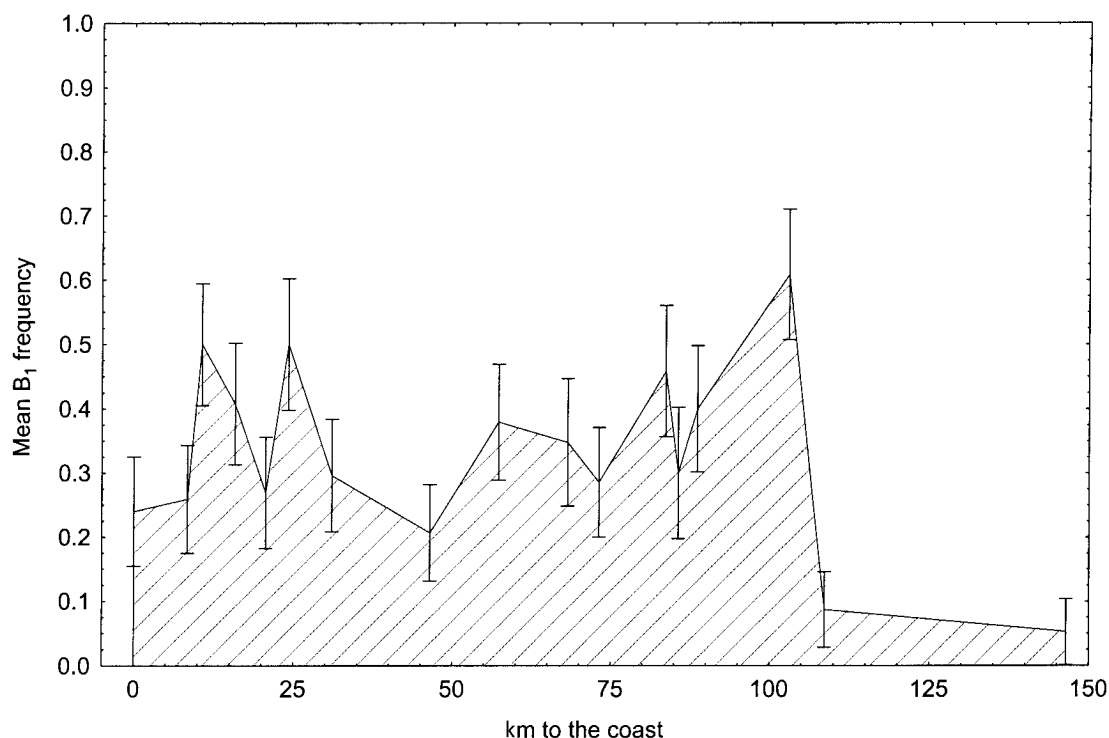


Fig. 5. Mean B₁ frequency along the Segura River transect in 2001. Error bars represent \pm one standard error.

IBD, except for the mean distance of the last geographic distance class, which could be explained because the low sample size at that scale.

Valencia coastal populations

Twenty-three populations were sampled in a transect parallel to the Valencia coast, in a north-south direction. In this transect, two geographic landmarks separate north and south populations: the city of Valencia and La Albufera lake. These populations did not show significant differences in B distribution (χ^2 contingency test = 86.05, $P = 0.516$) and did not show significant association between population differences in prevalence, load or mean frequency with the geographic distance between them (Mantel tests, $r = -0.045$, $P = 0.256$; $r = 0.049$, $P = 0.198$; $r = -0.006$, $P = 0.509$).

The distograms showed several significant departures from the expected random distribution of distances for 10 km intervals (at 20–30, 30–40 and 50–60 classes, see Fig. 6), but these departures were removed when a 20-km interval was used. This result seems to be more robust than the first one, at 10-km scale, since sample size (i.e. pairs of data per distance class) increases when a longer interval distance is used.

Turia River populations

Ten populations were sampled in 1995 in a longitudinal transect along the Turia River, starting 54 km upstream from the last population closest to the city of Valencia. These populations showed significant differences among them (contingency χ^2 test = 65.73, $P < 0.001$), but this was not reflected in the

result of the Mantel test comparing the matrix of geographic distances along the river with the matrices of dissimilarity in prevalence ($r = 0.225$, $P = 0.082$), load ($r = -0.141$, $P = 0.235$) and mean B frequency ($r = 0.231$, $P = 0.088$).

The distogram of 10-km intervals showed no significant departures from random distribution (Fig. 7) but a slight trend to increased genetic distance with geographic distance. The distogram of 15 km classes showed that the populations separated by 0 to 15 km were more similar than expected by chance ($P = 0.032$) and, again, there was a positive association between geographic and genetic distances.

Jucar River populations

Twelve populations were sampled along 50 km of the course of this river in 1996. These populations also showed significant differences in the distribution of individuals with different numbers of Bs (contingency χ^2 test = 49.94, $P < 0.028$). As in the case of the Turia River, there was no significant relationship between the matrix of geographic distances and prevalence ($r = -0.058$, $P = 0.373$), load ($r = -0.189$, $P = 0.075$) or mean B frequency ($r = -0.073$, $P = 0.333$) dissimilarity matrices.

Distograms showed a general non-significant trend toward increased differences in B frequency with distance, except at the 30–40-km interval where populations were more similar than expected ($P = 0.002$ and $P = 0.014$, respectively; see Fig. 7). As this result seemed exceptional, we calculated the distogram considering the real position of these populations in a two-dimensional space, since the Jucar River has a turn that could explain the previous results: distant populations along

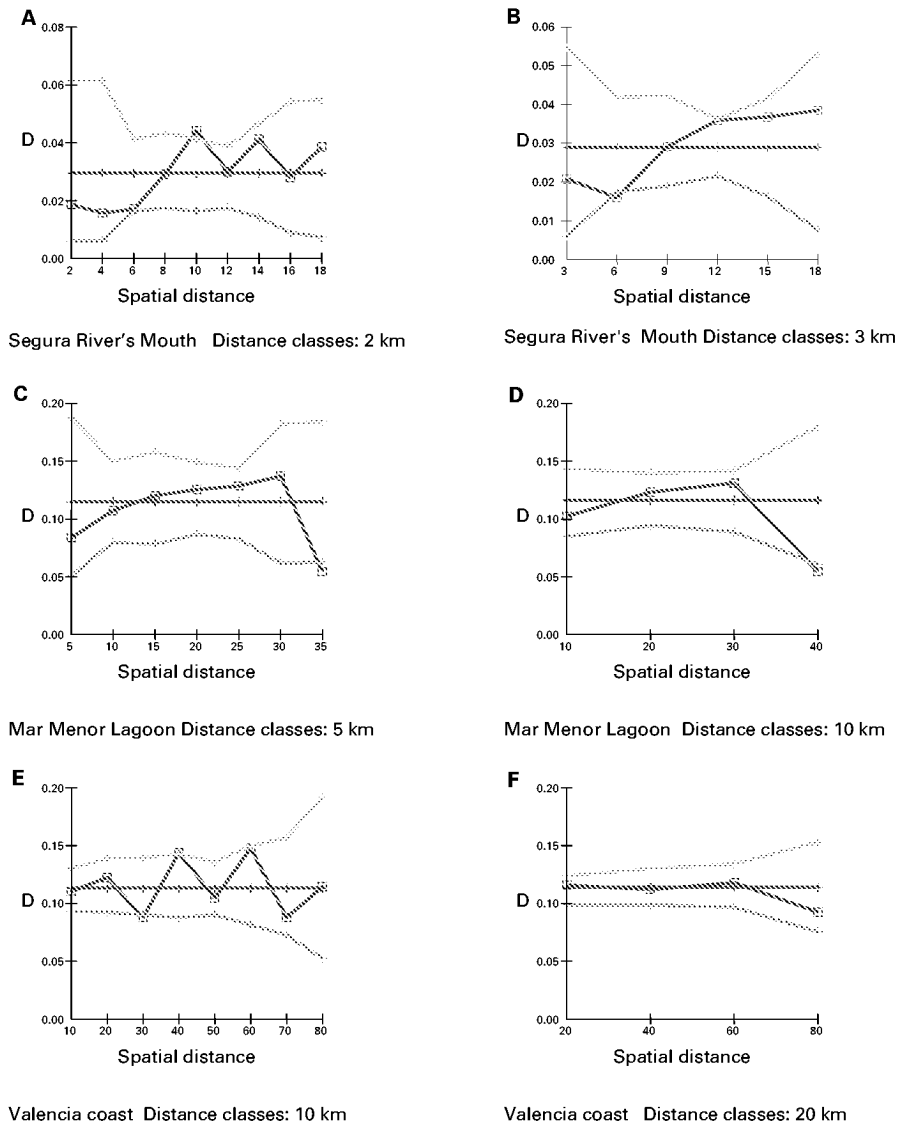


Fig. 6. Distograms for Gregorius distances for Segura River mouth, Mar Menor Lagoon and Valencia coast transects.

the river course may be closer in a “as the bird flies” line. When we calculated the new distogram, the previous significant positive structure was lost.

Discussion

The contingency analysis of the populations in each transect showed that only in the river transects there were significant differences in the distribution of individuals with different numbers of Bs among populations. The populations in transects at low altitude (closest to the coast) showed more similarity than the high-altitude populations. If this similarity is produced by a higher rate of gene flow between these populations, this could not be determined without a geographic explicit approach.

We have used two geographic explicit approaches to analyze B chromosome frequency in these populations. They have

advantages and caveats. Mantel tests can show directional trends, but they may not detect non-linear variations. Correlograms and distograms can be used with confidence if there is a high number of pairs of data in each distance class (Degen, 2000), a situation that was not always possible to fulfill because of limitations in the number of populations per transect. In addition, genetic distances among populations with the larger geographic separations should not be considered nor interpreted because they are based on only a small number of findings, i.e. the most distant populations (Epperson, 1993; Legendre and Legendre, 1998).

We have used three frequency parameters to account for the variability of B chromosome frequency: prevalence, load and mean frequency. Of these, load seems to be the less sensitive to variations, probably due to its small range. In fact, the Mantel test between load and geographic distance matrices only showed significant results in two years in the Segura River transects, and the signs of these correlations were different.

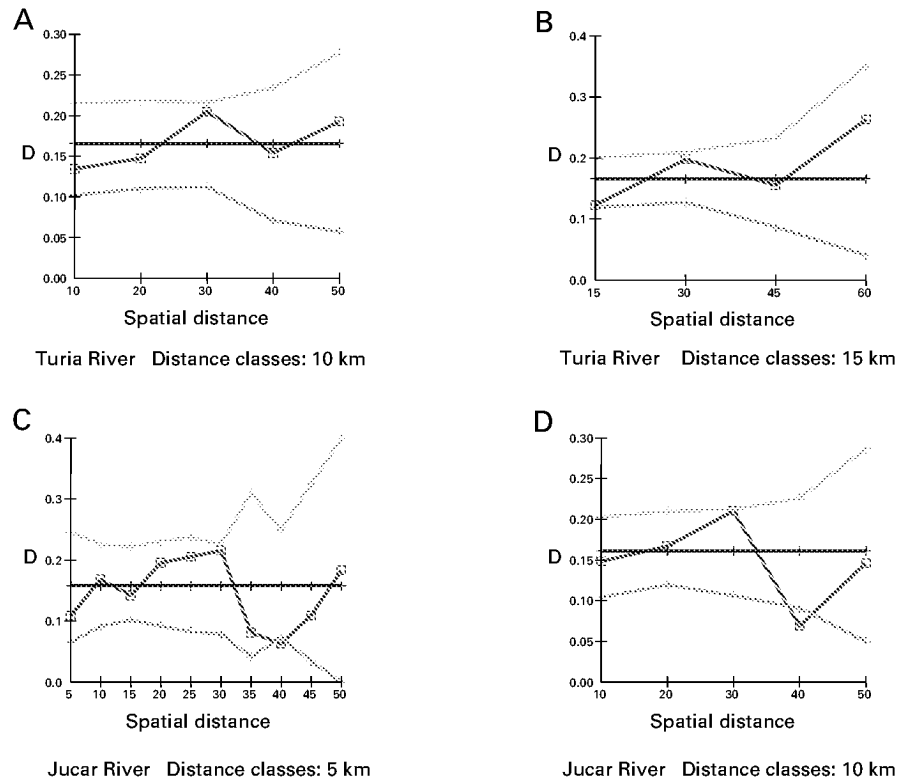


Fig. 7. Distograms for Gregorius distances for Turia and Jucar Rivers.

It is known that the prevalence of a neutralized B chromosome does not show ample variations (Camacho et al., 1997); thus, it could only be used to detect high differences between populations, as it seems to be the case in the Segura River transects.

The mean frequency of the B chromosome seems to be the best parameter to characterize the frequency of the B chromosome polymorphism. In fact, it incorporates the combined information of both prevalence and load since the mean is equal to the product of prevalence and load.

Different processes can produce the observed pattern of proximal populations showing higher similarity, e.g. high gene flow (migration), recolonization, descent from the same population, or local adaptation. Low migration and drift, however, could produce isolation by distance. The populations at the Segura River mouth have shown the most appealing case for IBD (see Fig. 6B for a typical representation of this process). At short geographic distances, populations are more similar than at longer distances. In addition, this trend was also manifested by the Mantel test for mean frequency of Bs. This pattern appeared for populations separated by only a few kilometers, implying that migration levels are not so important to homogenize the frequency of the B₁ chromosome, but sufficient to produce IBD. However, due to low sample size (in terms of populations), this result should be considered with caution.

With the increased anthropic fragmentation of natural habitats and following risk of loss of some habitats, migration should probably be reduced (Hanski, 1999) and the effects of genetic drift would be expected to increase, without however producing a clear spatial pattern because of the stochasticity of

this process. Low gene flow could also contribute to the increase of variation among populations. However, the gene flow necessary to prevent substantial population differentiation is very low (Hanski, 1999), since values as low as $N_e m = 1$, i.e. one individual per generation per year, are sufficient to prevent local differentiation at bidimensional spaces for some theoretical models (Maruyama, 1970, 1971). These homogenization effects of migration could explain the absence of significant differences among populations, at least at some coastal transects. Other possible explanations for the absence of spatial structure in some transects (e.g. Valencia coast) is that populations at high geographic scales of analysis are showing independent stochastic variation (drift) that is saturating the variation range of this variable. Sokal and Oden (1991) have shown that autocorrelations for shorter distances are stronger and overall less variable than when larger distance scales of analysis are used. Stochastic variation is greater for larger distance classes (Epperson, 1993), and this stochasticity could produce non significant or random distogram.

The populations at the Turia River were significantly non-homogeneous, but Mantel tests failed to reveal any linear pattern. In fact, the samples for two nearby populations at the mid-course of the river showed no B chromosomes, which could produce the significant result in the contingency test. The distograms of these populations, and those for the Jucar River transect, could also be compatible with IBD, although no significant departures from random variation have been found, which is usual with low sample size.

In the Segura River transects, we have not found evidence for a temporal trend, which is consistent with the slow changes

expected from the long random walk for neutralized B chromosomes such as B₁ (Camacho et al., 1997). But these transects revealed a clear spatial pattern, with B₁ showing a clinal variation, with lower values of prevalence and mean frequency in the upper course of this river. This pattern was detected by the multiple regression analysis and also the Mantel test. In fact, in the headwaters of this river there are several populations without B chromosomes, which are probably isolated from the downstream populations by physical barriers (Cabrero et al., 1997). The analysis of the distograms showed although not conclusively that, in recent years, the more distant populations were more different in B₁ frequency. Gene flow between upper- and lower-course populations could be reduced leading to local differentiation because several reservoirs built along the Segura River could contribute to reducing the number of migrants between both groups of populations. In addition, distance classes around 80 km, in some years (i.e. 1993, 2000 and 2001, see Fig. 3 and 4) showed significant increases in genetic distance between pairs of populations. At this spatial scale, we are actually comparing the upper and lower populations, i.e. populations with high and low frequency of B chromosomes.

This variation could be produced by genetic drift and reduced gene flow or by local selection, but also could reflect, at least partially, effects of selection and drive acting in the past. As Epperson (2003) remarks, IBD for neutral genes can often produce similar pattern as selection at some spatial scales. Unfortunately, we do not have unequivocal data to discriminate between these two hypotheses. The evolutionary dynamics

expected for a neutralized B chromosome (Camacho et al., 1997) is mainly determined by a random walk caused by the loss of B drive. But, since B chromosomes do not show a regular meiotic behaviour (i.e. they do not go in segregating pairs as A chromosomes), they cannot be fixed by genetic drift. Since some selection against individuals with a high number of Bs is expected, the random walk is thus biased towards B extinction. Another reason why random walks for neutralized Bs are expected to be protracted comes from a metapopulation perspective (multiple populations with some gene flow among them), since computer simulation analyses have shown that it prolongs very much the duration of this stage of B chromosome life cycle (Camacho et al., 1997). The present results provide the first empirical evidence of IBD in the evolution of a neutralized B chromosome, and it is appropriately explained by the metapopulation approach. In addition, the existence, in the Segura River, of some samples lacking Bs surrounded by B-carrying samples, which in later samplings showed B presence, point to the possible importance of recolonization in the population dynamics of *E. plorans*.

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