Non random distribution patterns of supernumerary segments and B chromosomes in *Dichroplus elongatus* (Orthoptera)

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Dichroplus elongatus is a grasshopper, which exhibits parallel polymorphisms for B chromosomes and supernumerary segments in chromosomes S_{10} (SS10), S_9 (SS9) and M_6 (SS6) in natural populations of Argentina. The patterns of simultaneous variation of different forms of supernumerary heterochromatin from 7 populations of two biogeographic provinces located at Northwest and East regions of this country were analysed. Spatial chromosome differentiation for all heterochromatic variants was observed. Different degree of differentiation among populations for supernumerary segments, evaluated through F_{ST}, was observed. This suggests that genetic drift and migration may not be the only factors involved in the maintenance of the detected chromosome patterns. The differentiation for SS9 and SS10 may be explained mainly by heterogeneity within the Northwest Region. On the contrary, the differences for SS6 and B chromosomes explained through isolation by distance. However, the absence or the low frequency of both chromosome mutations in some populations may not be explained by historical factors. A principal component analysis showed that the patterns of chromosome variation do not agree with the geographical distribution of the populations. The relationship between frequencies of each supernumerary segment and B chromosome depends on the segment. An analysis of partial correlation showed that the frequencies of B chromosomes were positively correlated with the frequency of SS6 and negatively with the frequency of the SS10. In agreement with this, a multiple regression analysis of B chromosome frequency on supernumerary segment frequencies depends on the incidence of SS6 and SS10. The covariation of the heterochromatic forms may act as another factor that establish limits to stochastic factors, may also affect the probable negative selection on B, and could be involved in the non random pattern detected in *D. elongatus*.

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The heterochromatic elements of natural populations of animals and plants vary extensively. Some heterochromatic variants are integrated into the genome as C-bands (VOSA 1976; PORTER and RAYBURN 1990), knobs (LONGLEY 1938) or supernumerary segments (HEWITT 1979; JOHN 1983). Another form of heterochromatic variation is related with the presence of B or supernumerary chromosomes. These B's often exhibit accumulation mechanisms which involve preferential segregation at meiosis or nondisjunction at gametophyte mitosis with preferential inclusion in the functional gametes, and less frequently mitotic instability, preferential fertilisation and paternal genome elimination (JONES and REES 1982; BEUKEBOOM 1994). B's are in general considered as 'selfish', since they apparently do not produce any adaptive phenotypic effects on carriers, and their main activity is related to their transmission (MATTHEWS and JONES 1982).

Heterochromatic forms integrated into the normal complement offer, however, limited opportunities for non Mendelian segregation. Within Orthoptera there are rather few examples of such accumulation. One of them is the case of *Eyprepocnemis plorans*, in which supernumerary segments may be associated with distortional segregation during meiosis (LÓPEZ LEÓN et al. 1991).

When different forms of supernumerary heterochromatin are present in the same population, interactions among them may be observed (RHOADES and DEMPSEY 1973; LÓPEZ LEÓN et al. 1991). Such covariation may affect the pattern of distribution of different kinds of supernumerary heterochromatin.

Dichroplus elongatus is a South American grasshopper that exhibits parallel polymorphisms for supernumerary chromosomes and segments in natural populations of Argentina (LORAY et al. 1991; CLEMENTE et al. 1994). Both forms of supernumerary heterochromatin are associated with effects on chiasma frequency and distribution. An altitudinal cline in the incidence of B chromosome has been found (REMIS and VILARDI 1986; LORAY et al. 1991; CLEMENTE et al. 1994; SEQUEIRA et al. 1995).

Here supernumerary chromosome and segment frequencies were jointly analysed in seven populations from two different biogeographic provinces from Argentina. We test here whether there are interactions among the different forms of supernumerary heterochromatin and analyse the pattern of simultaneous variation and the levels of differentiation reached for each chromosome mutation within and between two main geographic regions.

MATERIAL AND METHODS

Samples of adult grasshoppers were collected from 7 localities of two biogeographic provinces of Argentina = 'Las Yungas' and 'Pampeana'. Raco (RA), Tafí Viejo (TV), Horco Molle (HM), Famaillá (FA), Campo Quijano (CQ) belong to 'Las Yungas' and are

located in the Northwest of Argentina. Ingeniero Maschwitz (IM) and San Clemente (SC) are located at Pampeana Province in the East region of this country (Fig. 1).

A total of 448 males were analysed cytogenetically between 1982 and 1998 (Table 1).

Cytogenetic analysis

Male testes were dissected out, fixed and conserved in 3:1 (absolute alcohol:acetic acid) at 4°C. The cytological preparations were made by squashing follicles in propionic haematoxylin.

Data analysis

The patterns of spatial variation were analysed employing the results of the present work and data from previous studies (Table 1). In all cases, where χ^2
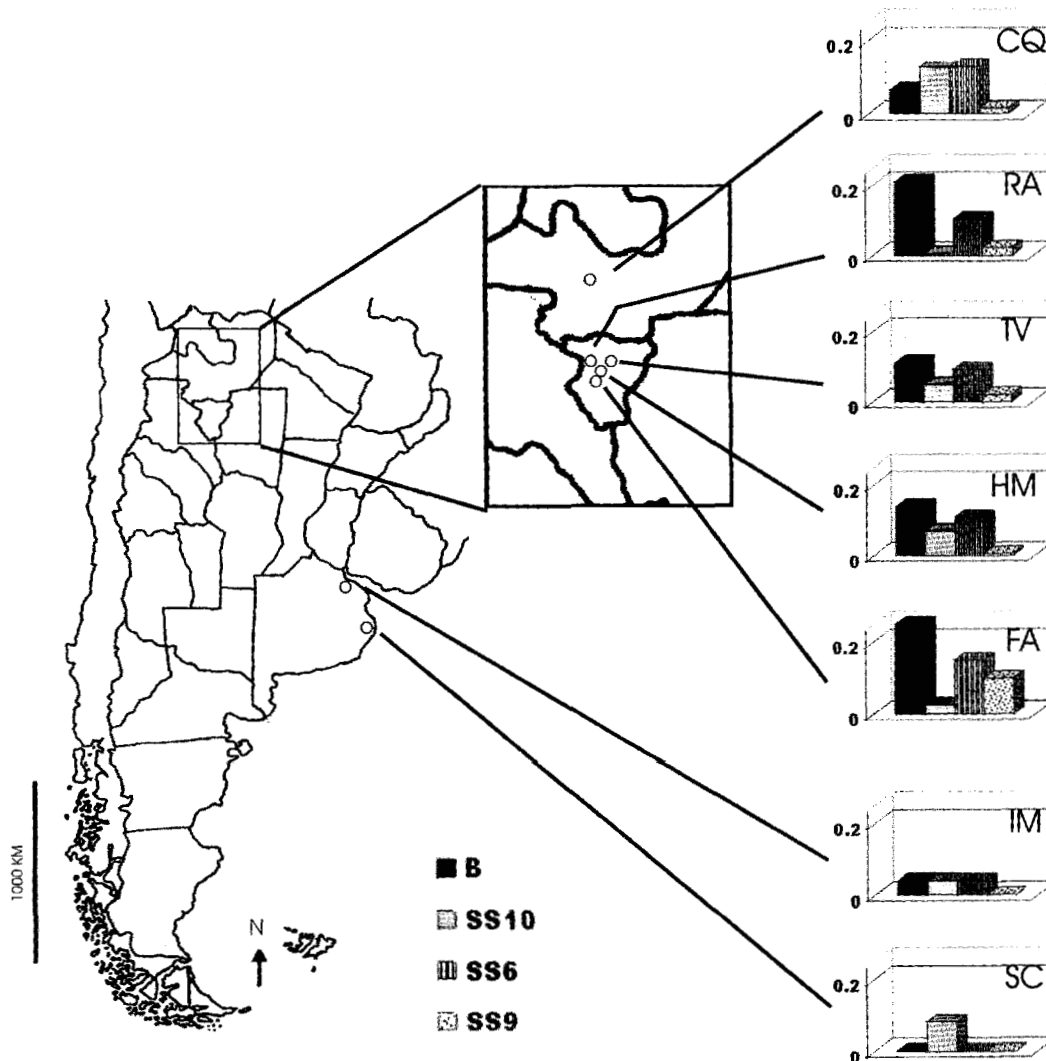

Fig. 1. Map showing the localities where the populations of *Dichroplus elongatus* were sampled and the relative incidences of each form of supernumerary heterochromatin. CQ = Campo Quijano, RA = Raco, TV = Tafí Viejo, HM = Horco Molle, FA = Famaillá, IM = Ingeniero Maschwitz, SC = San Clemente.

Table 1. *Frequencies of B-chromosomes (B) and supernumerary segments in S10 S6 and S9 pairs (SS10, SS6 and SS9 respectively) from natural populations of Dichroplus elongatus collected in Argentina between 1982 and 1998*

Population	Year	N	B	SS10	SS6	SS9
RACO	1982*	23	0.174	0.000	–	0.022
	1990	30	0.100	0.000	0.066	0.016
	1992 #	38	0.316	0.013	0.132	0.026
	Average		0.209	0.005	0.103	0.022
TAFI VIEJO	1987**	42	0.095	0.048	0.107	0.036
	1988***	20	0.250	0.025	0.100	0.000
	1990	60	0.083	0.041	0.050	0.008
	1992 #	31	0.096	0.081	0.145	0.032
	Average		0.111	0.049	0.092	0.020
H. MOLLE	1990	52	0.173	0.077	0.115	0.009
	1992 #	27	0.074	0.019	0.093	0.000
	Average		0.139	0.070	0.108	0.006
FAMAILLA	1993	20	0.250	0.025	0.150	0.100
C. QUIJANO	1996	10	0.000	0.100	0.100	0.000
	1998	21	0.095	0.143	0.143	0.024
	Average		0.065	0.129	0.129	0.016
I. MASCHWITZ	1992 #	25	0.040	0.040	0.040	0.000
S. CLEMENTE	1984 # #	49	0.000	0.082	0.000	0.000

* REMIS and VILARDI 1986.
** LORAY et al. 1991.
*** CLEMENTE et al. 1994.
  # SEQUEIRA et al. 1995.
REMIS 1989.

contingency tests can be made, the frequencies of each heterochromatic variant did not differ among years. Accordingly, the data of all samples were pooled for each population to provide suitable population sizes.

Chromosome differentiation for supernumerary segments was evaluated using F-statistics (WRIGHT 1951, 1965) estimated according to Weir and Cockerham's method (WEIR and COCKERHAM 1984) and through a hierarchical analysis of population differentiation according to WRIGHT (1978). Both indices were calculated employing the BIOSYS 1.7 program (SWOFFORD and SELANDER 1981). In some cases, negative F_{xy} values were obtained since, as SWOFFORD and SELANDER (1981) pointed out, negative variance components sometimes result when total variance is partitioned orthogonally into estimated variance components. The statistical significance of chromosome differentiation was performed through χ^2 contingency test. This method was limited to the populations with sample sizes high enough as to avoid faultfinding cells (cells with expected frequency lower than 5, in more than 10% of cells) (cf. COCHRAN 1952, 1954).

The relationships between B and heterochromatic segment frequencies were analysed by means of partial correlations. To evaluate the relative importance of each supernumerary segment frequency, multiple regression (stepwise selection procedure) was per-

formed. For these parametric studies, the data were transformed according to CHRISTIANSEN et al. (1976).

To analyse the data of all heterochromatic variants frequencies, the populations were also represented in orthogonal axes from principal components analysis (PCA) which also avoids some of the multicolinearity between the frequencies.

The analysis of partial correlation, multiple regression and PCA were made with the STATISTICA program (STATISTICA STATSOFT Inc. 1996).

RESULTS

Natural populations of *Dichroplus elongatus* from Argentina are polymorphic for mitotically unstable B chromosomes and supernumerary segments in M6 (SS6), S9 (SS9) and S10 (SS10) pairs (see LORAY et al. 1991; CLEMENTE et al. 1994).

In the present work, the parallel variation of these chromosome polymorphisms, through 7 populations belonging to two different biogeographic provinces from Argentina, was analysed (Table 1) (Fig. 1).

There is an apparent heterogeneity among populations in the frequency of B chromosomes (Table 1). When regions are compared after pooling data of all populations within each region, the contingency table yielded significant values ($\chi_1^2 = 9.81$, P = 0.01). The analysis of among population differentiation within

Table 2. F_{ST} *coefficients for supernumerary segments and the Chi-square values to test their significance. (Contingency tests have applied only in cases with suitable sample sizes, see Material and Methods)*

	SS10	SS6	SS9
Among populations			
F_{ST}	0.022	0.014	0.019
χ^2	11.60	12.52	
P	0.02	0.01	
Among Northwest populations			
F_{ST}	0.027	−0.005	0.018
χ^2	9.44	0.35	−
P	0.009	0.83	−
Between East populations			
F_{ST}	$<10^{-3}$	−0.005	−
Between regions			
F_{ST}	$<10^{-3}$	0.045	0.009
χ^2	1.80	11.40	−
P	0.18	0.0007	−

Northwest region was limited to three populations of highest sample size and suggested homogeneity for B chromosomes within this region ($\chi^2_2 = 4.4$, P = 0.11). Though in the East region a similar test could not be properly done both populations are quite similar, so that B chromosomes are absent or have an extremely low frequency (see Table 1). Accordingly, the heterogeneity in B chromosome frequencies among populations may be explained by a significant differentiation between regions.

Chromosome differentiation, employing the data of supernumerary segments, was evaluated through F_{ST} coefficients (see Materials and Method) (Table 2). Significant and, in some cases highly, significant differences in the frequency of supernumerary segments were detected among populations. They show a clear spatial differentiation (Table 2).

The significant chromosome differentiation among populations in SS6 frequencies may be explained by the significant heterogeneity between biogeographic provinces since homogeneity was observed within Northwest region (Table 2). Even though the same

statistical comparison cannot be done within East (due to the limitations of χ^2 test), the estimated F_{ST} value in this region was rather high and similar to that in between region comparison. This would suggest that differentiation within East regions may also contribute to the total spatial heterogeneity.

For SS10 frequencies significant differentiation among populations may be primarily due to the heterogeneity among populations within the Northwest region (Table 2).

The analysis of F_{ST} coefficients for SS9 suggests a similar pattern of chromosome differentiation to the one observed for SS10. There is a higher differentiation among all populations and within the Northwest region. Unfortunately, contingency table tests could not be done due to the low frequency of this chromosome variant.

A hierarchical analysis of population differentiation using the data of supernumerary segments confirms the above results (Table 3). All SS6 chromosome heterogeneity is mainly explained by the between region component. For the remaining segments differentiation takes place only within the Northwest region.

In order to assess the simultaneous patterns of chromosome variation, the frequencies of heterochromatic variants were jointly analysed. As a common feature, the different heterochromatic forms are not independent. The presence of one type may affect the frequency of other forms of heterochromatin in the population. In the different samples, the frequency of SS6 segment increases and SS10 decreases with the incidence of B chromosomes (Table 1, Fig. 1). The analysis of partial correlation of transformed data showed that the frequency of B chromosomes was positively correlated with the frequency of SS6 and negatively with the frequency of SS10 (Table 4).

The stepwise multiple regression analysis of B chromosome frequency on supernumerary segment frequencies depends on the incidence of SS6 and SS10 (b = 0.728,   p = 0.009;   b = − 0.602,   p = 0.01 respectively).

Table 3. *Hierarchical F-statistics for supernumerary segments and the variance components*

	SS10		SS6		SS9	
	Variance component	F_{XY}	Variance component	F_{XY}	Variance component	F_{XY}
Among populations	0.0020	0.020	0.0034	0.021	0.0013	0.029
Among populations within region	0.0033	0.031	0.000	0.000	0.0015	0.033
Among regions	−0.0013	−0.012	0.0034	0.021	−0.0002	−0.005

Table 4. *Partial correlations between the different forms of supernumerary heterochromatin*

	B	SS10	SS6	SS9
B	1	−0.89*	0.88*	0.13
SS10		1	0.80	−0.05
SS6			1	0.15
SS9				1

* Significant differences.

The relationship between each supernumerary segment and B chromosome frequencies depends on the segment under consideration. In agreement with this, the frequency of supernumerary segments (pooling the data of all three segments) is not correlated with the incidence of B chromosomes ($r = 0.50$, $p = 0.25$).

The data on all heterochromatic variants was used in a principal component analysis (PCA). The three main components accounted for about 97.1% of the total variance in frequencies (Fig. 2). The first component, which explains for about 87% of the total variance, is affected by all heterochromatic variants in a similar way. The second and third components, which account for nearly 7 and 3%, respectively, of the total variance are mainly affected by B (positively) and SS10 (negatively) frequencies. In this picture, the populations are not always clustered as the geographic distances between them would suggest. Most strikingly, the East populations are very close to each other, while Northwest populations show a relatively high heterogeneity. Some Northwest populations are closer to East populations than to the rest of the populations in the same region.

DISCUSSION

The South American grasshopper *Dichroplus elongatus* exhibits spatial chromosome differentiation, and patterns of simultaneous variation for all heterochromatic variants were evident.

The among population differentiation for SS6, evaluated through F_{ST} coefficients, may be related to the heterogeneity between main regions (Northwest and East) and the isolation by distance. On the contrary, for SS10 the chromosome differentiation may be most important among Northwest populations since no differences between regions were found. Similar F_{ST} results were found for SS9. The heterogeneity in the frequency of neutral chromosome mutations may be explained through genetic drift and migration. This would result in a similar degree of differentiation among populations for each chromosome. In our study, this simple hypothesis cannot explain the different levels of chromosome differentiation seen in SS9 and SS10 as contrasted to SS6. These results do not agree with the ones obtained through phylogeographic studies on supposedly neutral markers as mitochondrial DNA RFLPs (CLEMENTE et al. 1998). The mtDNA analysis showed highly significant differences between regions while the relationships among Northwest populations, based on Nei's genetic distances (considering haplotypic frequencies), seem to be better explained through biogeographic than simple geographic considerations. The Northwest populations located on the south-eastern edge of 'Las Yungas' province (HM and FA) showed homogeneity for mtDNA haplotype
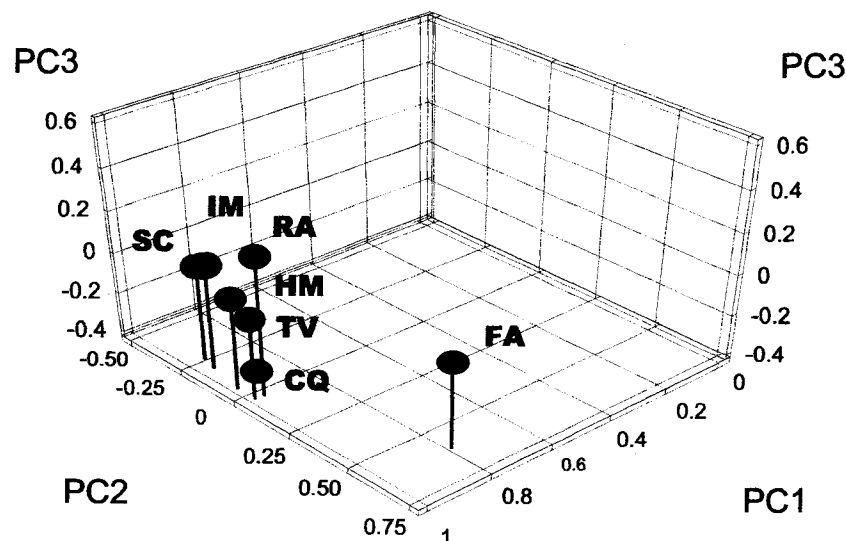
Fig. 2. Principal component analysis in polymorphic populations for supernumerary heterochromatin of *Dichroplus elongatus*. Tridimensional graphics of the three principal components (1, 2 and 3). They account for nearly 97.1% of the total variation in the frequencies of the different heterochromatic variants.

distribution and the same holds for CQ and RA, located on the western side of 'Las Yungas' (CLEMENTE et al. 1998). Accordingly, mtDNA results showed simultaneously heterogeneity between biogeographic provinces and within one of them. The chromosome differentiation among populations detected here for supernumerary segments does not agree with such molecular patterns.

The spatial B chromosome differentiation was similar to that observed for SS6 showing significant differences only between main regions. The principal component analysis (PCA) that incorporates the data of all heterochromatic variants showed that populations are not clustered according to their geographical distances. This may be due the fact that the distribution of SS6 and B chromosome polymorphisms differs from the differentiation of SS9 and SS10 and again does not agree with the molecular (mtDNA) pattern.

When different forms of supernumerary heterochromatin are present in a single population, interactions among them are usually observed (LONGLEY 1938; RHOADES and DEMPSEY 1973; TARDIF and MORISSET 1992; LÓPEZ LEÓN et al. 1991, 1994). LONGLEY (1938) reported a negative relation of knobs and B's in native maize. RHOADES and DEMPSEY (1973) showed that B chromosomes cause elimination of chromatin knobs-bearing members of the regular chromosome complement at the second microspore mitosis of maize. Similar results were obtained in the grasshopper *Eyprepocnemis plorans* in supernumerary chromosome bearing individuals. Here the distortion segregation of a supernumerary segment leads to a decrease in its transmission rate. This effect depends on the dosage of B (LÓPEZ LEÓN et al. 1991, 1994). In relation to the above examples of polymorphisms involving one or both types of heterochromatic variants within the superfamily Acridoidea, LÓPEZ LEÓN et al. (1991) concluded that the competition for transmission between B and supernumerary segment is unlikely to be a frequent characteristic of grasshoppers. Following this, TARDIF and MORISSET (1992) considered that only specific bands would be functionally related to B-chromosomes. They were working with an analysis of C-bands in *Allium schoenoprasum*.

In *D. elongatus*, the frequency of different supernumerary segments varies with the incidence of B chromosomes. The frequency of SS10 decreases and the incidence of SS6 increases with the frequency of B chromosomes. When the data were evaluated through multiple regression, the relationships of B chromosomes and supernumerary segments are mainly explained by the variation in SS6 and SS10. The correlation between B and SS6 frequencies are in

agreement with previous cytogenetic results which showed a tendency towards an excess of the B + SS6 karyomorph class in TV population (CLEMENTE et al. 1994). A similar test to analyse the incidence of B + SS10 could not be done in any population because none of them bear simultaneously both rearrangements in frequencies high enough as to have the minimal expected frequencies of all karyomorphs.

The frequencies of each heterochromatic variant of *D. elongatus* remain roughly the same in consecutive samples (LORAY et al. 1991; CLEMENTE et al. 1994). This suggests that the association between B and SS6 and SS10 seems to be temporally stable. Under a neutral hypothesis, the distribution of heterochromatic variants might be the consequence of gene flow among populations with different origins coupled with genetic drift generating differences in karyomorph frequencies. This explanation seems to be untenable since, as mentioned before, chromosomally homogeneous Northwest populations (i.e. for SS6 and B chromosomes) exhibited restricted gene flow estimated through mtDNA variation (CLEMENTE et al. 1998). Accordingly, the pattern of chromosome variation cannot be explained by historical factors only.

BENNETT (1987) proposed that the amount of heterochromatin may affect the minimum size and mass of the cell and limit the time needed to produce a similar cell. He argued that the phenomenon could have critical adaptive significance. The negative correlation between different forms of supernumerary variants would permit the maintenance of tolerable amount of heterochromatin without affecting cell division.

Our results show differences in the relationships of the different forms of heterochromatin evidenced in changing signs of correlation depending on the chromosome pair. The results suggest that the amount of heterochromatin 'per se' may not be regarded as a general feature affecting normal cell division, since the frequency of one heterochromatic variant (i.e. SS6) increases with the incidence of B chromosomes. Since the presence of one type of heterochromatin would limit random fluctuations in the frequency of other correlated variants, the interactions among heterochromatic forms detected in *D. elongatus* may affect the distribution patterns and produce distortions from chance events (interactions among mutation, genetic drift and migration). In most cases supernumerary segment polymorphisms are not associated with selective effects (JOHN 1983). We do not have any direct evidence of adaptive effects of any supernumerary segment. On the contrary, previous cytogenetic studies have suggested that B's in *D. elongatus* are associated with an increment in abnor-

mal sperm production which may affect the fertility of the carriers (LORAY et al. 1991). Since B polymorphism seems to be temporally stable, the occurrence of an accumulation mechanism (such as mitotic instability) to counterbalance the negative effect on fertility has been proposed (LORAY et al. 1991). The covariation of the heterochromatic forms detected in the present paper would act as another factor that establishes limits to stochastic factors. This covariation may also affect the probable negative selection on B's, and seems to be involved in the non-random pattern detected in *D. elongatus*.

ACKNOWLEDGEMENTS

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