

# Chromosomal evolution and comparative gene mapping in the *Drosophila repleta* species group\*

Alfredo Ruiz<sup>1</sup>, José María Ranz<sup>1</sup>, Mario Cáceres<sup>1</sup>, Carmen Segarra<sup>2</sup>,  
Arcadio Navarro<sup>1</sup> and Antonio Barbadilla<sup>1</sup>

## ABSTRACT

A review of our recent work on the chromosomal evolution of the *Drosophila repleta* species group is presented. Most studies have focused on the *buzzatii* species complex, a monophyletic set of 12 species which inhabit the deserts of South America and the West Indies. A statistical analysis of the length and breakpoint distribution of the 86 paracentric inversions observed in this complex has shown that inversion length is a selected trait. Rare inversions are usually small while evolutionary successful inversions, fixed and polymorphic, are predominantly of medium size. There is also a negative correlation between length and number of inversions per species. Finally, the distribution of inversion breakpoints along chromosome 2 is non-random, with chromosomal regions which accumulate up to 8 breakpoints (putative "hot spots"). Comparative gene mapping has also been used to investigate the molecular organization and evolution of chromosomes. Using *in situ* hybridization, 26 genes have been precisely located on the salivary gland chromosomes of *D. repleta* and *D. buzzatii*; another nine have been tentatively identified. The results are fully consistent with the currently accepted chromosomal homologies between *D. repleta* and *D. melanogaster*, and no evidence for reciprocal translocations or pericentric inversions has been found. The comparison of the gene map of *D. repleta* chromosome 2 with that of the homologous chromosome 3R of *D. melanogaster* shows an extensive reorganization via paracentric inversions and allows to estimate an evolution rate of ~1 inversion fixed per million years for this chromosome.

## *Drosophila* as a model for the study of chromosomal evolution

For more than 80 years *Drosophila* has been a model organism for studies of genetics and evolution (Hartl and Lozovskaya, 1995). Among the advantages that make *Drosophila* an almost ideal material for research on genome evolution are the following. *i*) A relatively small genome size compared with other

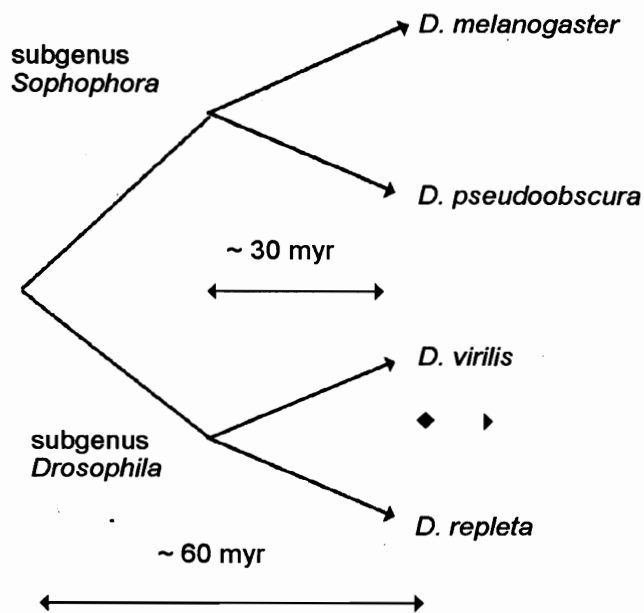
eukaryotes. The genome of *D. melanogaster* (165 Mb) is about one-twentieth the size of the human genome and harbors 15,000 to 20,000 genes with an average DNA content of 6 Kb (Merriam *et al.*, 1991; Hartl and Lozovskaya, 1995). *ii*) A vast amount of information gathered over the years about genes, mutations and chromosomal aberrations (Lindsley and Zimm, 1992; Flybase, 1997). *iii*) The presence of giant chromosomes in the salivary glands of the 3rd instar larvae (Sorsa, 1988). *iv*) Over 2,000 species classified in several subgenera and species groups (Wheeler, 1981) with a fairly well-known phylogeny (Powell and DeSalle, 1995; Figure 1).

Chromosomal evolution in the genus *Drosophila* may be investigated at three organization levels: cytological, genic and DNA. Cytological studies have traditionally focused on the salivary gland chromosomes whose banding patterns enclose an

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<sup>1</sup> Departament de Genètica i Microbiologia, Facultat de Ciències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain. Send correspondence to A.R.

<sup>2</sup> Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, 08071 Barcelona, Spain.



**Figure 1** - Phylogenetic relationships and approximate divergence times among four representative species of the genus *Drosophila* (modified from Hartl and Lozovskaya, 1995). *D. melanogaster* and *D. pseudoobscura* belong, respectively, to the *melanogaster* and *obscura* species groups of the subgenus *Sophophora*. *D. virilis* and *D. repleta* belong, respectively, to the *virilis* and *repleta* species groups of the subgenus *Drosophila*.

amazingly rich record of the species evolutionary history. By comparing these banding patterns among the members of a group of related species, it is possible to determine the number and kind of chromosomal rearrangements fixed during the species divergence since their last common ancestor, and to establish fairly elaborated chromosomal phylogenies (Sturtevant and Dobzhansky, 1936a,b; Stone, 1962; Wasserman, 1963). This approach has been extraordinarily fertile in ascertaining chromosome evolution *within* many species groups (see for reviews Ashburner *et al.*, 1982; Krimbas and Powell, 1992). On the other hand, the comparison of distantly related species belonging to *different* species groups is most often non-informative because the numerous changes occurred in the chromosome morphology make it difficult to confidently establish homology between chromosomal segments (Stalker, 1972; Yoon *et al.*, 1972; Wasserman, 1992).

The interspecific comparison of the linkage relationships of visible mutants and allozyme markers allows the study of chromosome evolution at the genic level (Sturtevant and Novitski, 1941; Patterson and Stone, 1952; Schafer *et al.*, 1993). Cytological and linkage map information led Muller (1940) to propose the hypothesis that the six chromosomal elements (named A through F) have been basically conserved in the genus *Drosophila* and to establish their putative homologies among various *Drosophila* species (Table I). The main shortcoming of using visible markers for comparative

**Table I** - Chromosomal homologies in the genus *Drosophila* (Muller, 1940; Papaceit and Juan, 1993). The dot chromosome has been omitted.

Species	Muller's element				
	A	B	C	D	E
<i>D. melanogaster</i>	X	2L	2R	3L	3R
<i>D. pseudoobscura</i>	XL	4	3	XR	2
<i>D. virilis</i>	X	4	5	3	2
<i>D. repleta</i>	X	3	5	4	2

purposes is that similar phenotypic changes can be produced by mutations at different loci and thus may not indicate true homology.

The study of chromosomal evolution at the DNA level requires the cloning of large genomic regions by chromosome walking and their posterior characterization in different species. So far the *Antennapedia* gene complex (*ANT-C*) is the largest chromosomal region analyzed in *Drosophila* using this scheme. Interspecific comparisons of the *ANT-C* organization show that, yet the basic structure of the complex has been conserved, some minor changes have occurred during divergence (Hooper *et al.*, 1992; Randazzo *et al.*, 1993; Terol *et al.*, 1995). In fact, the detection of such small changes is only possible in these comparative studies of accurate molecular maps along particular DNA regions. However, the difficulties to undertake these kind of studies at a large scale, covering complete chromosomal sections or even whole chromosomes, are obvious.

An alternative and feasible method to study chromosome evolution makes use of the *in situ* hybridization technique (Pardue *et al.*, 1970) which allows the localization on the polytene chromosomes of a given species of DNA sequences homologous to those included in recombinant clones obtained from the same or a different species. *In situ* hybridization using DNA clones has been used to test the chromosomal homologies proposed by Muller (1940) in several species groups (Steineman, 1982; Steineman *et al.*, 1984; Whiting Jr. *et al.*, 1989; Papaceit and Juan, 1993; Lozovskaya *et al.*, 1993) and, in a few cases, to compare the molecular organization of chromosomes among *Drosophila* species. Using both gene containing clones and P1 phages, Segarra and Aguadé (1992) and Segarra *et al.* (1995, 1996) have compared the molecular organization of Muller's elements A, D and E between *D. melanogaster* and six species of the *obscura* group also included in the subgenus *Sophophora*. Likewise, relying on both genetically mapped markers (Gubenko and Evgen'ev, 1984) and *in situ* hybridization data (Whiting Jr. *et al.*, 1989; Lozovskaya *et al.*, 1993), Kress (1993) has

compared the organization of chromosomal elements A and D between *D. melanogaster* and *D. virilis*, two species which belong to different subgenera (Figure 1). These studies have revealed that the investigated chromosomal elements have experienced a profound reorganization, but remarkably they have also led to the identification of a few small chromosomal segments which have seemingly been conserved during the long time span since the species divergence.

A review of our recent studies on the chromosomal evolution of the *Drosophila repleta* species group is presented here. These include the classical interspecific comparisons of the banding patterns of salivary gland chromosomes but also a thorough statistical analysis of the physical length and breakpoint distribution of all the paracentric inversions described in the *Drosophila buzzatii* species complex. In addition, a summary of our ongoing mapping studies using *in situ* hybridization is provided. We end up with a global discussion of our results and prospects for future research.

### Chromosomal evolution of the *Drosophila buzzatii* complex

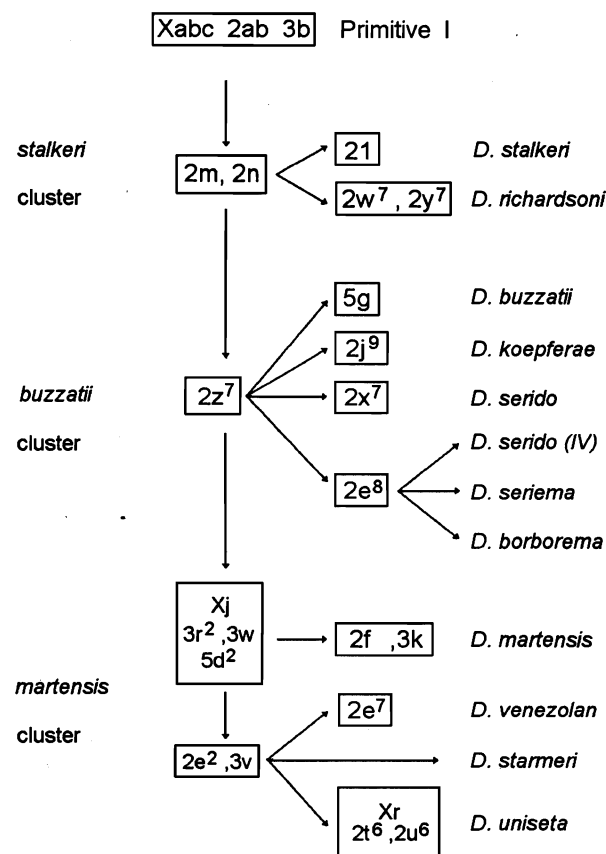
The *repleta* group is one of the largest species groups in the genus *Drosophila*, comprising over ninety species assigned to five subgroups (Wasserman, 1982, 1992). The karyotype of most *repleta* species consists of five telocentric chromosomes and a dot chromosome (Table I) and is thus similar to the putative ancestral karyotype of the genus (Muller, 1940; Clayton and Guest, 1986). The salivary gland chromosomes of many of the species have been compared to those of *D. repleta* (Wharton, 1942) and a fairly complete inversion phylogeny has been built (Wasserman, 1982, 1992). Very likely, the ancestral arrangement of the *repleta* group is not that of *D. repleta* (the reference species for cytological studies) but one, designated as Primitive I, that differs from it by at least six paracentric inversions, *Xa Xb Xc 2a 2b 3b* (Wasserman, 1982, 1992). Paracentric inversions are by far the prevalent type of chromosomal change within the *repleta* group; rearrangements involving heterologous chromosomes are rare (only four centric fusions have been described) and pericentric inversions seem entirely absent (Wasserman, 1982, 1992). Chromosome 2 contains about 23% of the euchromatin but harbors 208 out of the 296 inversions (70%). It is thus the most dynamic and phylogenetically most informative (Wasserman, 1992).

Our studies have focused on the *buzzatii* species complex defined by Ruiz and Wasserman (1993) as a set of ten closely related species belonging to the *mulleri*

subgroup and distributed through the deserts of South America and the West Indies. These ten original species were *D. buzzatii*, *D. koepferae*, *D. serido*, *D. boroborema* (comprising the *buzzatii* cluster), *D. martensis*, *D. starmeri*, *D. venezolana*, *D. uniseta* (which make up the *martensis* cluster), *D. stalker* and *D. richardsoni* (included in the *stalker* cluster). Another species, *D. seriema*, has subsequently been included in the *buzzatii* cluster (Tidon-Sklorz and Sene, 1995). In addition, the populations of *D. serido* from Central-Western Brazil, which differ morphologically and chromosomally from the rest of the *D. serido* populations, very likely represent a separate, and yet undescribed, species (*D. serido* IV; Tosi and Sene, 1989; Silva and Sene, 1991). Therefore, the *buzzatii* complex currently comprises a total of 12 species.

The salivary gland chromosomes of *D. buzzatii*, *D. martensis* and *D. stalker* were first analyzed by Wasserman (1954, 1962) who derived them as independent lineages from Primitive I. However, further studies on these and other subsequently discovered species (Ruiz *et al.*, 1982; Ruiz and Wasserman, 1993) have shown unequivocally that these three species (and consequently their respective clusters) are closely related. Chromosome 2, which is the most informative, differs by a single inversion ( $2f^2$ ) between *D. buzzatii* and *D. martensis* and by two inversions only ( $2l$  and  $2z^7$ ) between *D. buzzatii* and *D. stalker* (Ruiz and Wasserman, 1993). Figure 2 shows an updated version of the most parsimonious chromosomal phylogeny, established by comparing the banding patterns of the various species and also by the observation of the polytene chromosomes in many interspecific hybrids (Ruiz and Wasserman, 1993; Kuhn *et al.*, 1996). Spicer (1995) has recently constructed a molecular phylogeny of eight of the twelve species of the *buzzatii* complex by sequencing the mitochondrial cytochrome oxidase subunit I, II and III genes. His results are fully congruent with the proposed inversion phylogeny (Figure 2). The results of the comparative mapping study reported below (Ranz *et al.*, 1997) are also consistent with this phylogeny.

Nineteen paracentric inversions have been fixed during the evolution of the *buzzatii* complex species from the ancestral arrangement Primitive I. Two inversions ( $2m$  and  $2n$ ) are shared by all species in the complex. One more inversion ( $2z^7$ ) has been fixed in the species of the *buzzatii* and *martensis* clusters and another four ( $Xj$ ,  $3r^2$ ,  $3w$  and  $5d^2$ ) are homozygous in the *martensis* cluster only. In addition, most of the species have their own characteristic homozygous inversions. Three inversions ( $2f^2$ ,  $2e^7$  and  $2t^6$ ) are fixed in one species but apparently are still segregating in a different



**Figure 2** - Chromosomal phylogeny of the *Drosophila buzzatii* species complex. Only the paracentric inversions homozygous in each of the species are shown (updated from Ruiz and Wasserman, 1993).

one. A total of 67 inversions have been described as intraspecific variation in the 12 species of the *buzzatii* complex. Ruiz and Wasserman (1993) classified these inversions in two classes according to their evolutionary success: polymorphic (observed in at least two different localities) and rare inversions (found in a single locality only). The number of polymorphic inversions per species varies between zero in *D. stalker* and 14 in *D. starmeri* with an average of 3.6. The number of rare inversions per species varies between zero in *D. stalker*, *D. venezolana* and *D. uniseta*, and eight in *D. buzzatii*, with an average of 2 (Ruiz and Wasserman, 1993).

## Length and breakpoint distribution of paracentric inversions

A complete statistical analysis of the length and breakpoint distribution of the 86 inversions described so far in the *buzzatii* complex (Ruiz and Wasserman, 1993; Kuhn *et al.*, 1996) has been recently carried out by Cáceres *et al.* (1977). In the following a summary of the most significant findings is provided. The paracentric inversions found in the *buzzatii* complex are not randomly distributed among the five major chromosomes, as there is a significant accumulation of inversions in chromosome 2 (Table II). Nevertheless, no association between chromosome and evolutionary success was observed: fixed, polymorphic and rare inversions show a similar distribution among the chromosomes. By contrast, the association between length and evolutionary success is highly significant (Table II). Fixed inversions are usually of medium size whereas rare inversions are predominantly small; polymorphic inversions, on the other hand, are more variable with representatives in the three length classes (Table II).

The observation that inversion length is associated with evolutionary success prompted a more detailed analysis of the length distribution in our data set. Seven inversions observed only once (unique) in the extensively studied species *D. buzzatii* (Ruiz *et al.*, 1984) were separated from the rest of rare inversions. Also, polymorphic inversions were sorted into widespread and endemic according to their presence in more or less than one fourth of the sampled localities. Figure 3 shows the length distribution in the resulting five classes of naturally occurring inversions. A sample of inversions induced in *D. buzzatii* by introgressive hybridization with its relative *D. koepferae* (Naveira and Fontdevila, 1985) has also been included for comparison. If the survival probability of an inversion were independent of its length, then the distributions of inversions with different degree of success would all be random samples of the same length distribution of newly-

**Table II** - Paracentric inversions described in the *Drosophila buzzatii* species complex classified according to evolutionary success, chromosome and length (Cáceres *et al.*, 1977).

Evolutionary success	Chromosome					Length			Total
	X	2	3	4	5	Small	Medium	Large	
Fixed	2	11	4	0	2	5	14	0	19
Polymorphic	2	32	4	2	3	14	21	8	43
Rare inversions	1	18	3	0	2	20	2	2	24
<b>Total</b>	<b>5</b>	<b>61</b>	<b>11</b>	<b>2</b>	<b>7</b>	<b>39</b>	<b>37</b>	<b>10</b>	<b>86</b>

occurring inversions. This is not the case in our data set as Figure 3 clearly illustrates. Induced and unique inversions, which may perhaps represent more closely the distribution of newly arising inversions, show a wide range of sizes. In contrast, most rare and endemic inversions are small, and the more successful inversions (polymorphic widespread and fixed) are predominantly intermediate in size. The differences among the various classes are highly significant (Cáceres *et al.*, 1977).

The species was also considered as another factor that might influence inversion length. Significant differences among species were detected for polymorphic inversions but not for rare inversions. Since the number of polymorphic inversions varies widely between species (see above), inversion length was further tested against the number of inversions per species. A significant negative correlation was observed. Species with many polymorphic inversions tend to have smaller inversions than those with fewer inversions and this correlation seemingly explains the differences observed between species.

For the study of the distribution of inversion breakpoints, chromosome 2, which harbors most of the inversions, was divided into 74 equal-length segments and the number of breakpoints in each segment was then scored (Figure 4). The analysis was carried out for all inversions pooled, and also separately for fixed, polymorphic and rare inversions. The distribution of breakpoints in the total sample departs significantly from the Poisson distribution with too many segments showing no breakpoints at all and a few segments containing too many (up to eight) breakpoints. A few particular bands (C6a, D1g, D5a, E2e, F6a and F2a) seem to accumulate a strikingly disproportionate number of breaks (Figure 4). On the other hand, when the different samples were tested separately, only fixed inversions showed a significant departure from Poisson. The breakpoint distributions of polymorphic and rare inversions did

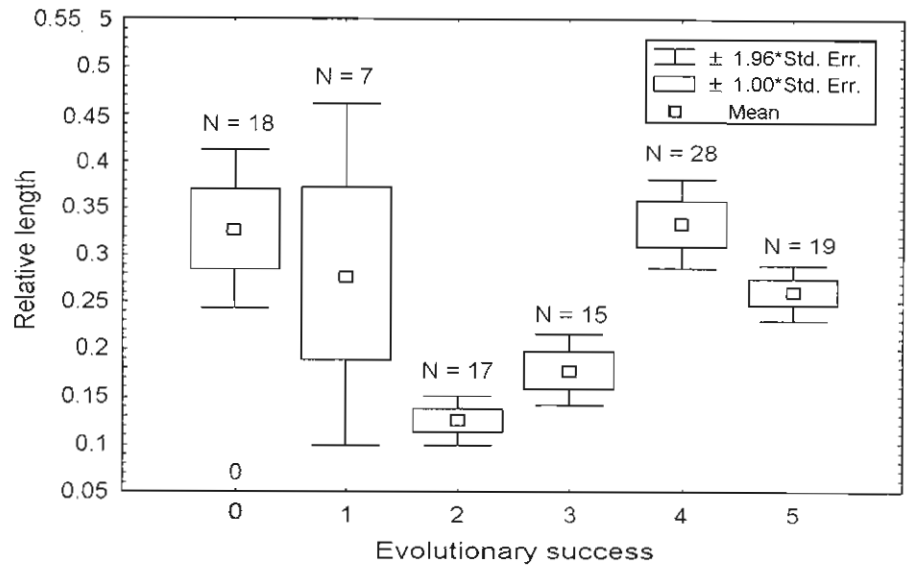


Figure 3 - Length distribution of the chromosomal inversions described in the *Drosophila buzzatii* complex. Evolutionary success: 0 = inversions induced in the laboratory by introgressive hybridization between *D. buzzatii* and *D. koepferae* (Naveira and Fontdevila 1985); 1 = unique inversions; 2 = rare inversions; 3 = polymorphic endemic inversions; 4 = polymorphic widespread inversions, and 5 = fixed inversions. See Cáceres *et al.* (1977) for details.

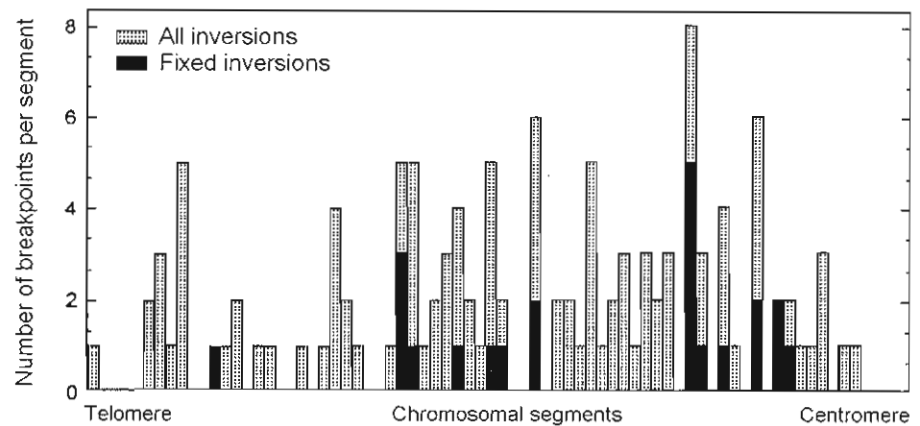


Figure 4 - Breakpoint distribution of the chromosome 2 inversions. Note the presence of several apparent "hot spots" with a disproportionate number of breaks (data from Cáceres *et al.*, 1977).

not depart separately from the Poisson distribution, but they tended to coincide.

### Cytological gene mapping in the *Drosophila repleta* group

So far, only a limited number of genes have been studied in the *repleta* group species. Partial linkage maps of visible mutants and allozyme loci are available for three species only, namely *D. mulleri* (Spencer, 1957), *D. hydei* (Hess, 1976) and *D. buzzatii* (Schafer *et al.*, 1993; Betrán *et al.*, 1995). Based on this information, the probable homologies of the chromosomes of the *repleta* species with those of *D. melanogaster* have been established (Table I). In addition, a handful of genes have been physically mapped on the polytene

chromosomes of *D. hydei*, *D. repleta* and/or *D. buzzatii* using *in situ* hybridization or other techniques (see Ranz *et al.*, 1997, for a review). In general, these data have supported the proposed chromosomal homologies although some exceptions have been reported (Ranz *et al.*, 1997).

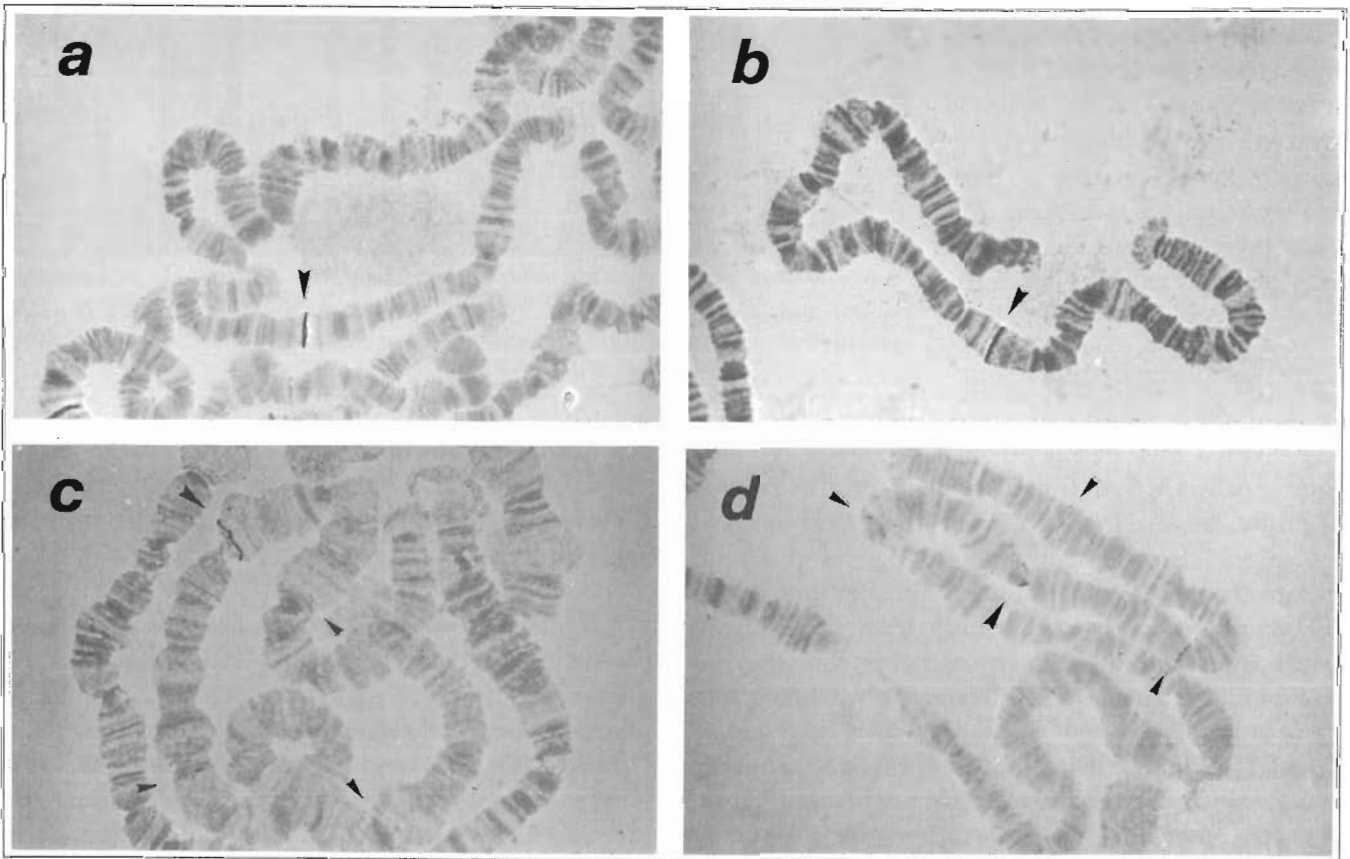
We have undertaken a big mapping project aiming to locate by *in situ* hybridization on the polytene chromosomes of *D. repleta* and *D. buzzatii* a large number of protein-coding genes previously cloned in *D. melanogaster* and/or other *Drosophila* species. So far 33 DNA clones have been assayed. The results are given in detail elsewhere (Ranz *et al.*, 1997); only a brief account will be presented here. Twenty-six out of the 33 clones (79%) produced one or more hybridization signals (Table III). Only seven probes did not produce detectable hybridization signals after several attempts. When a single and consistent signal was observed (17 probes), this undoubtedly must correspond to the site of the gene homologous to that present in the clone. As an example, Figure 5a,b shows the hybridization signal observed with the clone of the gene *Delta* (*DI*). Note that the hybridized band is identical in *D. repleta* and *D. buzzatii*, although its position on the chromosome is not the same due to a fixed inversion (2*b*) between the two

species. *DI* was the only gene in our sample for which previous linkage information (in *D. buzzatii*) was available (Schafer *et al.*, 1993) and, as expected, the physical and genetic localizations were concordant.

When multiple signals were produced (9 probes), one of them (the primary signal) was usually more intense than the rest and was interpreted as pointing the position of the homologous gene in the two *repleta* group species. The additional (secondary) signals could often be indirectly matched to known genes of *D. melanogaster* using four kinds of clues: *i*) the relative intensity of the various hybridization signals; *ii*)

**Table III** - Number of gene probes hybridized to the salivary gland chromosomes of *D. buzzatii* and *D. repleta* classified according to their expected chromosomal localization and the number of hybridization signals produced (Ranz *et al.*, 1997).

Hybridization signals	Chromosome					Total
	X	2	3	4	5	
None	0	6	0	1	0	7
Single	0	15	1	1	0	17
Multiple	0	5	1	2	1	9
Total	0	26	2	4	1	33



**Figure 5** - Hybridization signals produced by the clone of the gene *Delta* in (a) *D. buzzatii* and (b) *D. repleta*, and by the clone of the gene *Hsp70B* in (c) *D. buzzatii* and (d) *D. repleta*. Large arrowheads point to primary hybridization signals; small arrowheads to additional (secondary) signals. (Taken from Ranz *et al.*, 1997).

the proposed chromosomal homologies between *D. melanogaster* and *D. repleta* (Table I); *iii*) the additional information gathered from previous mapping studies of the *repleta* group species; and *iv*) the close proximity of the secondary signals to other gene markers of known localization. An appropriate example will be discussed here. Five hybridization signals were observed with the clone of *heat shock protein 70B* (*Hsp70B*): four on chromosome 2 (Figure 5c,d) and one on chromosome 4. Based on signal intensity and previous information (Peters *et al.*, 1980) two of the chromosome 2 signals were identified as *Hsp70* and *Hsp68*. Another chromosome 2 signal was ascribed to *Hsc70-2* due to its proximity to another mapped gene, *Xdh* (Livak *et al.*, 1978). Finally, the other two signals were tentatively assigned to *Hsc70-4* and *Hsc70-1* relying on the chromosomal homology with *D. melanogaster*.

In our study, twenty-six autosomal genes were directly localized on the polytene chromosomes of *D. repleta* and *D. buzzatii*: 20 on chromosome 2, three on chromosome 4, two on chromosome 3 and one on chromosome 5. Besides, another nine genes were indirectly mapped thanks to the secondary signals and the complementary information: five on chromosome 2, two on chromosome 4 and two on chromosome X. Therefore, a total of 36 gene markers were mapped, if the secondary signal of the gene *tailless* (*tll*) is included. The localization of the 27 gene markers mapped on chromosome 2 is shown in Figure 6.

## Applications of comparative mapping

Besides the basic genetic information provided by this study, the mapping data allow five main applications.

1) *Test of chromosomal homologies*. In all cases our results are fully consistent with the proposed chromosomal homologies between *D. repleta* and *D. melanogaster* (Table I) and in no case evidence for reciprocal translocations or pericentric inversions has been found.

2) *Mapping genes and inversion breakpoints*. When a gene is located near one breakpoint of a

polymorphic inversion, the relative position of both may be more accurately defined by comparing the hybridization signal of that gene in the standard and inverted arrangements. For instance, it has been shown in this way that the *Pp1-96A* gene is located inside inversion 2j but outside inversion 2z<sup>3</sup>, both polymorphic in *D. buzzatii*. This in turn shows that the distal breakpoints of both inversions are not precisely identical as previously reported (Wasserman, 1962; Ruiz *et al.*, 1984).

3) *Evolution of gene families and gene complexes*. The homeobox gene family (Gehring and Hiromi, 1986; Ruddle *et al.*, 1994) is distributed in *D. melanogaster* in two separated gene complexes: the *Antennapedia* complex (*ANT-C*) at 84B1-2 (Kaufman *et al.*, 1990) and the *Bithorax* complex (*BX-C*) at 89E1-2 (Duncan, 1987). We hybridized one gene clone from each complex to the chromosomes of *D. repleta* and *D. buzzatii*. The chromosomal sites of *Antp* and *Ubx* were coincident or adjacent in both species (Figure 6). The same result has been recently obtained in *D. virilis*, another species of the *Drosophila* subgenus (Von Allmen *et al.*, 1996). In the latter species, the *BX-C* is split between the *Ubx* and *abd-A* transcription units; *Ubx* is clustered with the *ANT-C* at one site (24E) of chromosome 2 whereas *abd-A*

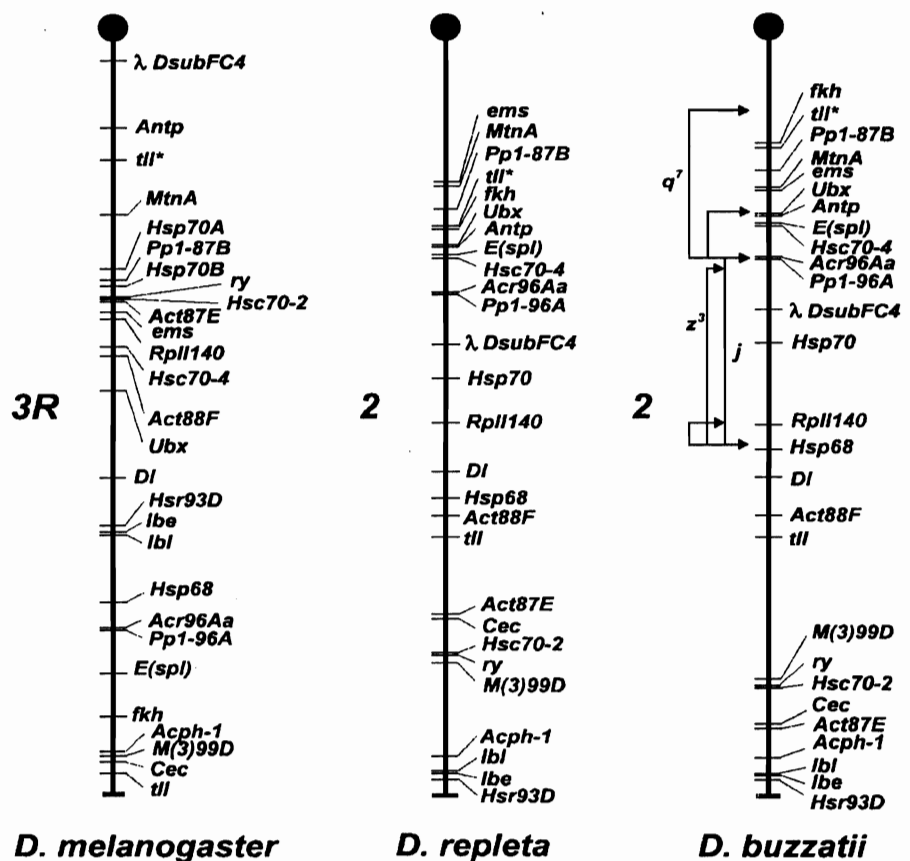


Figure 6 - Localization of the 27 gene markers available on chromosome 2 of *D. repleta* and *D. buzzatii*, and on the homologous chromosome 3R of *D. melanogaster* (from Ranz *et al.*, 1997).

and *abd-B* map at another site (26D) of the same chromosome (Von Allmen *et al.*, 1996). Thus, most likely, a single HOX gene complex was present in the ancestor of the genus *Drosophila*, as it is today in other insects like *Tribolium* (Ruddle *et al.*, 1994). This complex split differently in the two lineages leading to the *melanogaster* and the *virilis* species groups (Figure 1). More work is needed to determine whether in the *repleta* group the HOX genes are still together as in the ancestor of the genus or split in the same way as in *D. virilis*.

4) *Test of the cytological phylogeny.* The comparison of the gene order between *D. repleta* and *D. buzzatii* (Figure 6) allows a check of the proposed chromosomal phylogeny (Figure 2). Seven fixed differences between these two species have been detected on chromosome 2: *2a*, *2b*, *2t*<sup>8</sup>, *2u*<sup>8</sup>, *2m*, *2n* and *2z*<sup>7</sup> (Wasserman, 1992; Ruiz and Wasserman, 1993). Some of these inversions (but not all) are obvious when comparing the order of the 27 gene markers available on chromosome 2 between *D. repleta* and *D. buzzatii*. Thus, our comparative mapping results are consistent with the chromosomal phylogeny but not yet as informative as the direct comparison of the salivary gland chromosome banding patterns.

5) *Rate of chromosomal evolution.* The comparison of the gene arrangement of chromosomal element E between *D. melanogaster* and *D. repleta* shows an extensive reorganization (Figure 6). Most markers change their relative position along the chromosomes indicating that a large number of paracentric inversions have taken place since the divergence of the two lineages. The comparison of all possible pairs of contiguous markers on this element between *D. melanogaster* and *D. repleta* reveals that only three small chromosomal segments (*ry-Hsc70-2*, *Acr96Aa-Pp1-96A* and *lbe-lbl-Hsr93D*) have seemingly been conserved. Using a maximum likelihood procedure, we estimated that 130 paracentric inversions have been fixed between *D. melanogaster* and *D. repleta* in this chromosomal element. Taking 60 million years as the divergence time between the two subgenera (Figure 1), a rate of fixation of inversions in element E of 1 inversion per million years was obtained. This value is comparable to other estimates of chromosomal evolution in *Drosophila* (Table IV) but is much higher than that observed for mouse and man. These two species have diverged for about 70 million years and differ by 144 chromosomal rearrangements (mostly inversions and translocations). This gives a figure of 1 rearrangement per million years for the entire genome, which is about 20 times the

**Table IV** - Rates of chromosomal evolution (number of rearrangements fixed per million years) estimated by comparative mapping.

Comparison	Chromosome	Rate	Reference
<i>D. melanogaster</i> / <i>D. repleta</i>	3R	130/120	Ranz <i>et al.</i> , 1997
<i>D. melanogaster</i> / <i>D. virilis</i>	All	83/120	Nurminsky <i>et al.</i> , 1995
<i>D. melanogaster</i> / <i>D. pseudoobscura</i>	X	56/60	Segarra <i>et al.</i> , 1995
Man/mouse	All	144/140	Copeland <i>et al.</i> , 1995

genome of *Drosophila* and about 100 times the size of element E.

## Discussion and future prospects

Genome evolution deals with the patterns and mechanisms of change in genome size, structure and organization through time (Hartl *et al.*, 1995). In principle, the interspecific comparison of karyotypes and genome organizations provides information about patterns only and not about processes. Nevertheless, knowing which changes have actually taken place and which have not may motivate questions about mechanisms and suggest hypotheses for further tests. This kind of studies have played an important role in the past and will certainly continue to do so in the future.

Paracentric inversions are the only gross chromosomal rearrangements observed in the evolution of the *buzzatii* complex. This agrees with the cytological evolution of the *repleta* group (Wasserman, 1992), and also with that of the entire genus *Drosophila* (Krimbas and Powell, 1992). While over 28,000 paracentric inversions may be polymorphic in the extant *Drosophila* species and over 42,000 may have been fixed during the evolution of the genus, very few other rearrangements have been recorded (Stone, 1962; Sperlich and Pfriem, 1986; Papaceit and Juan, 1993). Reciprocal translocations are likely to be eliminated soon after their occurrence due to the semisterility of the heterokaryotypes (White, 1973). The absence of pericentric inversions may be attributed to two factors. On one side, most species in the *repleta* group have telocentric autosomes. Accordingly, pericentric inversions may arise only rarely due to the low probability of breaks at both sides of the centromere (none was detected by Naveira and Fontdevila, 1985, in their introgressive hybridization study). On the other hand, many pericentric inversions, but remarkably not all of them, cause a reduced fertility in their carriers (Coyne *et al.*, 1991, 1993). This effect is due to single and multiple crossovers within the inversion loop and, as expected, increases with the genetic length of the inversion (Navarro and Ruiz, *in press*).

In the *buzzatii* complex, the distribution of naturally occurring inversions among the five major chromosomes is clearly non-random, with a significant accumulation of inversions on chromosome 2. The same trend has been observed in the entire *repleta* group, the *melanica* species group (Wasserman, 1992) and the Hawaiian *Drosophila* (Carson, 1992). So far, no satisfactory explanation has been provided for this observation. The non-random distribution of inversions could be due to mutational and/or selective causes. Chromosome 2 (Muller's element E) is the larger one in the karyotype (Sturtevant and Novitski, 1941; Heino *et al.*, 1994) but the size difference is not enough for expecting a much higher mutation rate for this chromosome. A higher mutation rate would be expected if transposable elements, which have been implicated in the origin of chromosomal rearrangements (see below), were for any reason more abundant on chromosome 2. The inversions induced in *D. buzzatii* by introgressive hybridization with its relative *D. koepferae* (Naveira and Fontdevila, 1985) do not support this possibility. Terzagui and Knapp (1960) suggested that the presence of inversions in more than one chromosome pair may lead to a high degree of chromosome non-disjunction and production of unbalanced gametes, but their data in *D. pseudoobscura* do not unequivocally support their hypothesis. Thus, this is an open question.

A clear relationship between the length of inversions and their evolutionary success has been observed in the *buzzatii* complex. While newly arisen inversions seem to have a wide range of lengths, highly successful inversions are predominantly of medium size. This pattern is similar to that observed previously in other *Drosophila* species (Olvera *et al.*, 1979; Ruiz *et al.*, 1984; Krimbas and Loukas, 1980; Brehm and Krimbas, 1991; Krimbas and Powell, 1992) and can only be attributed to the operation of natural selection (Cáceres *et al.*, 1977). Paracentric inversions have many genetic effects, including the following. At the breakpoints, inversions may disrupt the nucleotide sequence of one or two genes or their continuity with regulatory sequences (Schneuwly *et al.*, 1987). They also alter the linear arrangement of genes, changing their chromosomal context. In the heterokaryotypes, inversions partially inhibit crossing-over between homologous chromosomes due to difficulties of synaptic pairing in the inverted segment or other reasons (Roberts, 1976; Coyne *et al.*, 1993). They also diminish fertility due to the production of unbalanced (inviable) gametes by crossing-over in the inverted region (Sturtevant and Beadle, 1936; Navarro *et al.*, 1977). Finally, inversions redistribute recombination. On one hand, they reduce it within and nearby the inverted regions (Navarro *et al.*,

1977), allowing the capture of favorable allelic combinations and the building up of coadapted gene complexes (Charlesworth and Charlesworth, 1973; Hartl, 1977). On the other hand, they can increase recombination in chromosomal regions outside the inversion (Grell, 1962; Luchessi and Suzuki, 1968; Navarro *et al.*, 1977). The higher evolutionary success of medium-sized inversions is likely due to two counteracting effects. On one side, the detrimental effect of paracentric inversions on fertility is expected to increase with the genetic length of the inversion (Navarro *et al.*, 1977) in a similar way as it does in pericentric inversions (Navarro and Ruiz, in press). On the other side, the probability of catching two genes with epistatic effects on fitness (or alternatively, the number of epistatic genes) is likely to increase with the size of the inversion. Therefore, only inversions with an intermediate size are big enough to enjoy the selective advantage of including epistatic genes and suffer but a negligible detrimental effect on fertility.

The apparent non-random distribution of breakpoints along chromosome 2 (Figure 4) is not surprising. It seems to hold true also when considering the whole *repleta* group, only 323 different breakpoints having been recorded for the 208 paracentric inversions described (Wasserman, 1992), and similar results have been observed in other *Drosophila* species (Krimbas and Loukas, 1980; Tonzetich *et al.*, 1988; Lemeunier and Aulard, 1992). The accumulation of breakpoints at certain "hot spots" may be due to the particular organization or nucleotide sequence of DNA at certain bands. It is tempting to attribute hot spots to the presence of transposable elements which induce chromosomal rearrangements in the laboratory (Engels and Preston, 1984; Collins and Rubin, 1984; Lim, 1988; Lim and Simmons, 1994). However, to date, the evidence of their implication in the origin of natural inversions is circumstantial (Lyttle and Haymer, 1992; Regner *et al.*, 1996) or negative (Wesley and Eanes, 1994; Cirera *et al.*, 1995). A cautionary note should be added. The apparent coincidence of breakpoints at the cytological level may fade out when studied in more detail, as our mapping data on the *Pp1-96A* gene show. Obviously, only future molecular studies of inversion breakpoints can resolve the issue.

Comparative gene mapping has a long history in the genus *Drosophila* (Sturtevant and Novitski, 1941; Patterson and Stone, 1952). The *in situ* hybridization technique, however, may take these studies into a new era. Our results in the *repleta* group have achieved a remarkable degree of success (almost 80% of the clones produced one or more hybridization signals) and are certainly very encouraging. As a first step, we plan to

increase the number of markers in chromosomes 2 and 4 (Muller's elements E and D). In order to reach an average density of one marker every 500 kb, 48 and 42 markers would be necessary in the two chromosomes which have a DNA content in *D. melanogaster* of ~24 and ~21 Mb, respectively (Heino et al. 1994). This seems to be a reasonable goal that would be made accessible for molecular studies almost any region of these chromosomes, including many inversion breakpoints.

Physical mapping by *in situ* hybridization has several applications as discussed above. Two of them will be emphasized here. Firstly, it allows to study the evolution of chromosomes at a resolution level intermediate between the classical cytology and the DNA sequencing projects. This may be sufficient to motivate new observations and interesting questions on the evolution of gene families and gene complexes, as our results on the homeobox gene family show. Secondly, it allows the comparison of the molecular organization in phylogenetically distant species. The comparison of element E between *D. melanogaster* and *D. repleta*, for instance, shows an extensive reorganization of this chromosomal element via paracentric inversions. This observation and those of other authors (Kress, 1993; Segarra et al., 1995, 1996) seem to indicate that the fact that paracentric inversions change the order of genes is not a crucial determinant of their evolutionary success. Their effect on crossing-over and recombination, as discussed above, seems to be a more important factor. The number of fixed inversion differences between two far-distant species may be estimated using mapping information by different methods (Nadeau and Taylor, 1984; Ranz et al., 1997). So far, the rather scarce available data (Table IV) suggest a much faster rate of chromosomal evolution in *Drosophila* as compared with mammals. In the future, the rates of chromosomal evolution will be estimated with increasing accuracy as the number of available markers increase. This will allow more reliable comparisons among diverse phylogenetic lineages.

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## RESUMO

Uma revisão de nosso recente trabalho sobre a evolução cromossômica do grupo de espécies de *Drosophila repleta* é apresentada. A maioria dos estudos refere-se ao complexo de espécies *buzzatii*, um conjunto monofilético de 12 espécies que habitam os desertos da América do Sul e das Índias Ocidentais. Uma análise estatística do comprimento e da distribuição do ponto de quebra de 86 inversões paracêntricas observadas neste complexo mostrou que o comprimento da inversão é um caráter selecionado. Inversões raras são geralmente pequenas, enquanto que inversões evolucionariamente bem sucedidas, fixas e polimórficas, são predominantemente de tamanho médio. Há também uma correlação negativa entre o comprimento e o número de inversões por espécie. Finalmente, a distribuição dos pontos de quebra nas inversões ao longo do cromossomo 2 não é aleatória, com regiões cromossômicas que acumulam até 8 pontos de quebra (possíveis "pontos quentes"). O mapeamento gênico comparativo também foi usado para investigar a organização molecular e a evolução dos cromossomos. Usando hibridização *in situ*, 26 genes foram precisamente localizados nos cromossomos da glândula salivar de *D. repleta* e *D. buzzatii*; outros 9 foram identificados por tentativa. Os resultados são completamente consistentes com as homologias cromossômicas correntemente aceitas entre *D. repleta* e *D. melanogaster*, não se encontrando evidências de translocações recíprocas ou inversões pericêntricas. A comparação do mapa gênico do cromossomo 2 de *D. repleta* com o do cromossomo homólogo 3R de *D. melanogaster* mostra uma extensa reorganização através de inversões paracêntricas e permite estimar uma taxa de evolução para este cromossomo de cerca de 1 inversão fixada por milhão de anos.

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