Chromosomal Elements Evolve at Different Rates in the Drosophila Genome

Josefa González, José María Ranz[1] and Alfredo Ruiz[2]

Departament de Genètica i de Microbiologia, Facultat de Ciències-Edifici C, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain

Manuscript received November 12, 2001
Accepted for publication March 21, 2002

ABSTRACT

Recent results indicate that the rate of chromosomal rearrangement in the genus Drosophila is the highest found so far in any eukaryote. This conclusion is based chiefly on the comparative mapping analysis of a single chromosomal element (Muller's element E) in two species, *D. melanogaster* and *D. repleta*, representing the two farthest lineages within the genus (the Sophophora and Drosophila subgenera, respectively). We have extended the analysis to two other chromosomal elements (Muller's elements A and D) and tested for differences in rate of evolution among chromosomes. With this purpose, detailed physical maps of chromosomes X and 4 of *D. repleta* were constructed by *in situ* hybridization of 145 DNA probes (gene clones, cosmids, and P1 phages) and their gene arrangements compared with those of the homologous chromosomes X and 3L of *D. melanogaster*. Both chromosomal elements have been extensively reshuffled over their entire length. The number of paracentric inversions fixed has been estimated as 118 ± 17 for element A and 56 ± 8 for element D. Comparison with previous data for elements E and B shows that there are fourfold differences in evolution rate among chromosomal elements, with chromosome X exhibiting the highest rate of rearrangement. Combining all results, we estimated that 393 paracentric inversions have been fixed in the whole genome since the divergence between *D. repleta* and *D. melanogaster*. This amounts to an average rate of 0.053 disruptions/Mb/myr, corroborating the high rate of rearrangement in the genus Drosophila.

CHROMOSOME repatterning is commonly thought to be of universal occurrence during the evolution of the eukaryotic genomes, even though only a few precise comparative analyses have been performed (Gale and Devos 1998; O'Brien *et al.* 1999; Seoighe *et al.* 2000; Ranz *et al.* 2001). Comparative mapping allows us to describe and estimate the amount of chromosomal evolution that has occurred during the divergence of species from their common ancestor, that is, the patterns and rates of genome reshaping (Nadeau and Taylor 1984; Nadeau and Sankoff 1998a; O'Brien *et al.* 1999). The amount of chromosomal evolution between two species can be expressed as the number of chromosomal rearrangements separating their genomes. Furthermore, by comparing the physical maps, segments in which the linear order of contiguous markers has been conserved can be identified. Closely related species are expected to have accumulated fewer rearrangements, and thus to share longer conserved segments, than distantly related species. Whether the particular gene combinations found in the conserved segments are preserved by natural selection, by structural DNA features that promote or restrict chromosome breakage, or simply by random processes is a matter of discussion (Hartl and Lozovskaya 1994; Nadeau and Sankoff 1998a; Huynen *et al.* 2001).

In the genus Drosophila, there is a remarkable synteny conservation; that is, the gene content of the five major chromosomal elements usually is preserved during the evolution of most lineages (Muller 1940; Sturtevant and Novitski 1941). This has allowed the establishment of homologies between the chromosomes of different species (Powell 1997). Per contra, the order of genes within each chromosomal arm is scrambled from species to species via the fixation of paracentric inversions (Hartl and Lozovskaya 1994; Segarra *et al.* 1995, 1996; Vieira *et al.* 1997a,b; Ranz *et al.* 1997, 1999, 2001; González *et al.* 2000), which are by far the most frequent chromosomal rearrangements in this genus (Krimbas and Powell 1992; Powell 1997). Exceptionally, a few cases of gene transposition have also been reported (Brock and Roberts 1983; Neufeld *et al.* 1991; Yi and Charlesworth 2000).

Remarkable differences in the rate of chromosomal evolution between phylogenetic lineages have been reported. In vertebrates, for instance, rates of synteny disruption vary >15-fold among lineages (Nadeau and Sankoff, 1998a; Murphy *et al.* 2001). Some vertebrate lineages (*e.g.*, human, carnivores, and common shrew) show remarkable conservation while others (*e.g.*, mice and the lesser apes) show extensive chromosomal re-

[1]*Present address:* Department of Organismic and Evolutionary Biology, Harvard University, D. L. Hartl Laboratory, Cambridge, MA 02138.

[2]*Corresponding author:* Departament de Genètica i de Microbiologia, Facultat de Ciències-Edifici C, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain. E-mail: Alfredo.Ruiz@uab.es.

arrangement (Ehrlich *et al.* 1997; Burt *et al.* 1999). However, the highest rate recorded so far in eukaryotes is that of Drosophila. Ranz *et al.* (2001) carried out a detailed comparative study of the largest chromosomal element (Muller's element E) between the species *Drosophila repleta* and *D. melanogaster*, representative of the two main lineages in the genus Drosophila. Their results revealed an extensive reshuffling of gene order from centromere to telomere and a rate of disruptions per megabase per million years two orders of magnitude higher than that of mammals and 5-fold higher than that of the most dynamic plant genomes. Only yeast genomes seem to exhibit rates of chromosomal rearrangement comparable (or perhaps greater) to those of Drosophila (Llorente *et al.* 2000; Seoighe *et al.* 2000). The between-lineages variation in evolution rate can be explained in terms of differential mutation rate, fluctuations of population size, variation in generation time, or differential fertility effects of chromosome rearrangements (Burt *et al.* 1999; Ranz *et al.* 2001).

Besides the variation among lineages, different chromosomes or chromosomal elements may also show unequal evolution rates. Rice (1984) pointed out that X-linked mutations with antagonistic effects in the two sexes should increase when rare under a much wider range of conditions compared to autosomal mutations. Moreover, Charlesworth *et al.* (1987) showed that the fixation rate of underdominant and advantageous partially recessive mutations should be higher for the X chromosome (due to the hemizygosity of males) than for the autosomes. For these reasons, the X chromosome has probably played a main role during the genetic differentiation associated with speciation. For example, in Drosophila, many hybrid sterility genes are X-linked (Orr 1997) and, in mice, the X chromosome harbors an unexpectedly large number of genes involved in sperm formation (Hurst 2001; Wang *et al.* 2001). Whether this functional specialization is related to the particular chromosomal dynamics of the X chromosome and the autosomes is unknown. In addition, X-linked genes undergo dosage compensation and the X chromosome must contain dispersed nucleotide sequences that act as a target for specific proteins and noncoding RNAs involved in this process (Kelley and Kuroda 1995; Stuckenholz *et al.* 1999). The autosomes may also exhibit variable evolution rates. Within several Drosophila species groups, such as the Hawaiian Drosophila or the *repleta* species group, the number of polymorphic and fixed paracentric inversions recorded in each chromosomal element is very unequal (Krimbas and Powell 1992). Also, using the comparative mapping approach, Vieira *et al.* (1997a,b) found different evolution rates between chromosomes within the *virilis* species group. So far, however, only relatively short-range phylogenetic comparisons have been carried out in the genus Drosophila.

We have investigated whether the chromosomal elements of Drosophila show nonhomogeneous evolution rates over long phylogenetic distances. Physical maps of the *D. repleta* chromosomes X and 4 have been constructed by *in situ* hybridization of 145 DNA clones (gene clones, cosmids, and P1 phages) and their gene arrangements compared with those of the homologous chromosomes X and 3L of *D. melanogaster* (Muller's elements A and D; see Figure 1). *D. repleta* belongs to the *repleta* species group of the Drosophila subgenus (Wasserman 1992) whereas *D. melanogaster* belongs to the *melanogaster* species group in the Sophophora subgenus (Powell 1997). They are representative species of the farthest lineages within the genus Drosophila, separated by 80–124 million years (myr; Figure 1). The aims of this study are (i) to estimate the number of inversions fixed in chromosomal elements A and D between *D. melanogaster* and *D. repleta*; (ii) to compare the evolutionary rates of these two chromosomes with those previously reported for Muller's elements E (Ranz *et al.* 2001) and B (González *et al.* 2000); and (iii) to shed light on the molecular organization of Drosophila chromosomes, find out conserved chromosomal segments, and test for functional constraints on the evolution of the Drosophila genome.

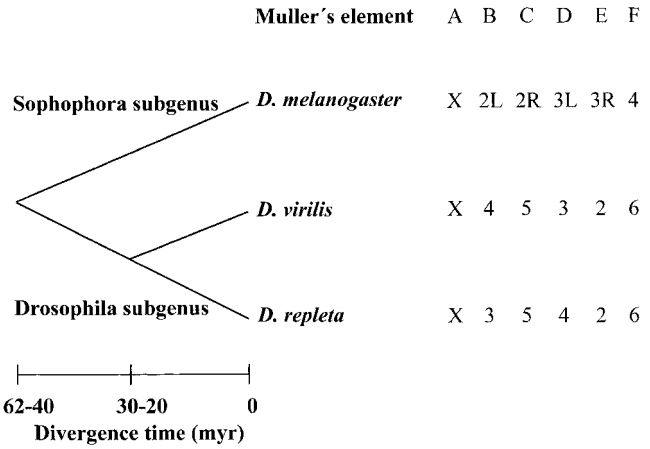

Figure 1.—Phylogenetic relationships and chromosomal homologies of the Drosophila species cited in the text. Estimates of the divergence time taken from Beverly and Wilson (1984), Spicer (1988), and Russo *et al.* (1995).

MATERIALS AND METHODS

Fly stocks: One stock of *D. melanogaster* (Canton-S), one stock of *D. repleta* (no. 1611.6 from the National *Drosophila* Species Resource Center, Bowling Green, OH), and one stock of *D. buzzatii* (39.13st) were used. The three stocks are homokaryotypic for the standard arrangement in all chromosomes (Lemeunier and Aulard 1992; Wasserman 1992; Ruiz and Wasserman 1993).

Probes: A total of 198 clones (46 gene clones, 64 cosmids, and 88 P1 phages) were used as probes. All these markers were previously known to map on chromosome X (111) or chromosomal arm 3L (87) of *D. melanogaster* (Table 1). Of the 46 gene clones, 14 are cDNAs from the *Drosophila melanogaster*

TABLE 1

Number of DNA clones successfully hybridized and number of clones assayed (in parentheses) to the polytene chromosomes of *D. repleta*

D. melanogaster chromosomal arm	Gene clones	Cosmids	P1 phages	Total	Source
X	20 (29)	46 (63)	8 (19)	74 (111)	This work
2L	—	—	26 (26)	26 (26)	González *et al.* (2000)
3L	15 (21)	1 (1)	58 (69)	74 (91)	This work; Ranz *et al.* (1997)
3R	63 (83)	48 (51)	43 (52)	154 (186)	Ranz *et al.* (2001)
Total	98 (133)	95 (115)	135 (166)	328 (414)	

Gene Collection (Rubin *et al.* 2000); 29 gene clones also come from *D. melanogaster* and were provided by different authors (Table 2); the remaining three gene clones (*Sod*, *sina*, and *Rh4*) were isolated by PCR in our laboratory and the PCR products cloned into a PGEM-T vector (Promega, Madison, WI) and partially sequenced to confirm their identity. The *Sod* fragment was amplified from *D. buzzatii* DNA according to the conditions and primers (N and O) reported for *D. melanogaster* (Hudson *et al.* 1994). The *sina* clone was produced from *D. buzzatii* DNA using primers (5′-GGAATTCCAGCTC TTCACTGTCGT-3′ and 5′-GGAATTCCCCAGTCGATAGAC AAA-3′) designed to match conserved *sina* nucleotide sequences between *D. melanogaster* and *D. virilis* (Neufeld *et al.* 1991). Finally, the *Rh4* clone was isolated from *D. virilis* DNA using primers (5′-GCCAAGTTGCTGTGCATT-3′ and 5′-ATC AGGCGGAGTTCGATT-3′) designed according to the *Rh4* nucleotide sequence of *D. virilis* (Neufeld *et al.* 1991). Cosmid clones come from the European *Drosophila* Genome Project cosmid library (Madueño *et al.* 1995) and P1 phages from the Berkeley *Drosophila* Genome Project P1 library (Hartl *et al.* 1994; Kimmerly *et al.* 1996). DNA from all these clones was extracted following standard procedures (Sambrook *et al.* 1989). Isopropyl thiogalactoside (0.1 m) was added to the overnight cultures of P1 phage clones (Hartl and Lozovskaya 1995). Cosmid clone 28C2 was digested with *Bam*HI and subcloned into pBluescript; four subclones were used as probes.

***In situ* hybridization and chromosome maps:** All clones were hybridized to the chromosomes of *D. repleta* to determine their physical localization in this species and to those of *D. melanogaster* as control. All hybridizations to the chromosomes of *D. melanogaster* gave positive results. In most cases, the hybridization signal was localized at the expected chromosomal site (appendix). However, two cosmids (156H1 and 13F10) and two P1 phages (DS08585 and DS00004) mapped to distant sites from those previously reported. We take this as an indication that these clones were probably mislabeled during the distribution process. Nevertheless, this does not diminish the utility of these clones as physical markers and they have been included accordingly in our marker set. In a few cases (see appendix) the map position of a marker in *D. repleta* was inferred from its localization in *D. buzzatii*, another species of the *repleta* species group (Wasserman 1992; Ruiz and Wasserman 1993). This can be safely done with markers mapping to homosequential chromosomal regions, *i.e.*, those regions not rearranged by paracentric inversions and thus with the same sequence of bands in the two species. Only female larvae were used for the hybridization of the X chromosome probes because the efficiency of hybridization on the female X is equivalent to that on the autosomes whereas the single X of the male shows a somewhat reduced level of hybridization (Pardue *et al.* 1987). Polytene chromosome squashes, hybridization, and detection were carried out as in Ranz *et al.* (1997). Probes were labeled with biotin-16-dUTP by nick translation. Hybridization signals were localized using the photographic maps of *D. melanogaster* polytene chromosomes (Lefevre 1976) and the cytological maps of *D. repleta* (Wharton 1942) and *D. buzzatii* (Ruiz and Wasserman 1993). Hybridization results were recorded as photographs taken with a phase contrast Nikon Optiphot-2 microscope at ×600 magnification. Examples of the hybridization results have been pictured in previous publications of our laboratory (Ranz *et al.* 1997, 1999, 2001; González *et al.* 2000).

Data analysis: Most of the genomic clones (cosmids and P1 phages) hybridized in this study have terminal sequence tagged sites (STSs; Hartl *et al.* 1994; Madueño *et al.* 1995; Kimmerly *et al.* 1996) that allowed us to localize them precisely on the genome sequence of *D. melanogaster* (Adams *et al.* 2000). When only one terminal STS was available, both the average size of each clone type [80 kb for P1 phages (Hartl *et al.* 1994) and 40 kb for cosmids (Madueño *et al.* 1995)] and its physical orientation were taken into account to anchor the clone in the genome sequence. The positions in the sequence of a few genomic clones with no STSs available were inferred from their cytological site (Hartl *et al.* 1994). Each pair of contiguous markers in the *D. melanogaster* (the reference species) map delimits a chromosomal segment of known size. All these chromosomal segments were checked for conservation in *D. repleta*. Those segments in which the relative order of contiguous markers is equivalent in both species were considered as conserved segments. Likewise, all clones yielding a single hybridization signal were considered as conserved segments ("singletons"). Otherwise, the segments were classed as nonconserved and assumed to bear at least one fixed inversion breakpoint. The maximum-likelihood method described in Ranz *et al.* (1997) was used to estimate the number of inversions fixed between the two species in each chromosomal element. This method does not require a particular distribution of markers along the chromosomes although it does assume random distribution of breakpoints in the reference species. The upper limit of the divergence time (Figure 1) was used to estimate the rates of evolution. Our evolution rate estimates are therefore conservative.

RESULTS

Positive hybridizations to the chromosomes of *D. repleta*: Nearly three-quarters of the assayed DNA clones (145/198 = 73.2%) yielded one or more hybridization signals on the chromosomes of *D. repleta* (or *D. buzzatii*; see materials and methods). The distribution of successful hybridizations by clone type and chromosome

TABLE 2

Gene clones hybridized in this study

Gene	Clone name	DNA	Insert size (kb)	Reference
ara	ara	cDNA	2.8	Gómez-Skarmeta *et al.* (1996)
arm	E9	cDNA	3.1	D. G. McEwen (personal communication)
BR-C	paaDm527	—	—	Andres *et al.* (1993)
caup	caup	cDNA	4	Gómez-Skarmeta *et al.* (1996)
csw	Y1.22	cDNA	—	Perkins *et al.* (1992)
CG1559	LD30316	cDNA	6.03	Rubin *et al.* (2000)
CG1716	SD01656	cDNA	4.94	Rubin *et al.* (2000)
CG3585	pDmY19S1b	Genomic	8	Kraemer *et al.* (1998)
CG4165	LD34905	cDNA	4.06	Rubin *et al.* (2000)
CG6450	SD02391	cDNA	4.68	Rubin *et al.* (2000)
CG7185	LD25239	cDNA	2.8	Rubin *et al.* (2000)
CG7282	LD25641	cDNA	5.11	Rubin *et al.* (2000)
CG7358	GH14795	cDNA	3.5	Rubin *et al.* (2000)
CG11056	LD44990	cDNA	3.98	Rubin *et al.* (2000)
CG12132	LD28902	cDNA	5.86	Rubin *et al.* (2000)
CG14616	GH02989	cDNA	3.7	Rubin *et al.* (2000)
CKIIalpha	pAPB21	cDNA	1.5	A. Bidwai (personal communication)
CKIIbeta	pAPB22	cDNA	0.94	A. Bidwai (personal communication)
ImpE1	IMPE2	cDNA	5.7	Natzle *et al.* (1986)
ImpE2	A2	Genomic	3.5	Natzle *et al.* (1986)
ImpL2	pL2G328	Genomic	2.6	Natzle *et al.* (1986)
ImpL3	pL23SE4	Genomic	4.2	Natzle *et al.* (1986)
Eip74EF	pE74AcDNA	—	—	Andres *et al.* (1993)
fog	SD02223	cDNA	4.58	Rubin *et al.* (2000)
Hsp83	301.1	Genomic	10.6	Holmgren *et al.* (1981)
Hsp22-Hsp26	88.6	Genomic	4.6	Córces *et al.* (1980)
Hsp23-Hsp27	88.5	Genomic	4.9	Córces *et al.* (1980)
Lsp1alpha	pR^{a}Lsp1	Genomic	8	McClelland *et al.* (1981)
msl-3	—	cDNA	1.9	Gorman *et al.* (1995)
peb	GH10905	cDNA	5.76	Rubin *et al.* (2000)
Pig1	pB′	—	0.75	Andres *et al.* (1993)
RpII215	LD43558	cDNA	5.01	Rubin *et al.* (2000)
rdgA	GH23785	cDNA	5.26	Rubin *et al.* (2000)
Rh4	PGPE110	Genomic	0.5	F. Casals (personal communication)
Rp21	pKc1G	Genomic	4.4	Kay *et al.* (1988)
sd	pNBE21	cDNA	3.2	Campbell *et al.* (1991)
sina	PGPE118	Genomic	0.6	F. Casals (personal communication)
Sgs4	pRH0.75	Genomic	0.75	McGinnis *et al.* (1983)
sisA	—	—	0.98	L. Sanchez (personal communication)
sn	P5	cDNA	3.3	Paterson and O'Hare (1991)
Sod	pGPE121	Genomic	1.9	This work
Yp1	pYP1	Genomic	3.8	Barnett *et al.* (1980)
Yp2	pYP2	Genomic	2	Barnett *et al.* (1980)
Yp3	pYP3	Genomic	3.9	Barnett *et al.* (1980)
w	pWXK	Genomic	4.8	O. Cabré (personal communication)
Zw	DmG21	Genomic	13	Ganguly *et al.* (1985)

is shown in Table 1, which also includes our previous results for clones from *D. melanogaster* chromosomal arms 2L (González *et al.* 2000), 3L (Ranz *et al.* 1997), and 3R (Ranz *et al.* 2001) for comparison. Overall, the rate of success is remarkably high (328/414 = 79.2%) given the long divergence time between *D. melanogaster* and *D. repleta* (Figure 1). There seem to be no differences between clone types ($G = 3.63$, d.f. = 2, $P > 0.05$) but there are highly significant differences between chromosomal elements ($G = 23.31$, d.f. = 3, $P < 0.001$). Chromosome X shows the smallest proportion of successful hybridizations (66.7%), significantly lower than that of the autosomes (83.8%; $G = 13.59$, d.f. = 1, $P < 0.001$). This difference seems to be due chiefly to cosmids ($G = 9.86$; d.f. = 1, $P < 0.01$) and P1 phages ($G = 17.05$, d.f. = 1, $P < 0.001$) rather than to gene clones, which show a similar hybridization rate ($G = 0.42$, d.f. = 1, $P > 0.05$).

All gene clones hybridizing to the chromosomes of *D. repleta* but one (*CG1716*; see below) gave a single

signal (APPENDIX). Likewise, most cosmid clones and P1 phages (98 out of 113) also gave a single hybridization signal (APPENDIX). Nevertheless, 15 cosmid clones or P1 phages gave two or more (up to four) hybridization signals in *D. repleta* chromosomes. These 15 genomic clones were considered to contain one or more (up to three) rearrangement breakpoints fixed during the divergence of *D. melanogaster* and *D. repleta*. This interpretation is supported by our previous results. Several cosmids and P1 phages giving multiple hybridization signals have been subcloned and the signals physically separated when the subclones were independently hybridized (RANZ *et al.* 1999; GONZÁLEZ *et al.* 2000). The present hybridization of two genes (*CG3585* and *Ubip5E*) included in cosmid clone 143G11 provides further evidence in favor of this interpretation. This cosmid gave two hybridization signals while each gene produced only one of them. Likewise, cosmid clone 28C2, giving three hybridization signals, was subcloned and hybridization of two of the subclones allowed us to physically separate two of the three signals. These results provide a firm basis for our interpretation of multiple signals as the result of the presence of fixed breakpoints in these genomic clones.

Thirteen of the gene clones are included in 10 of the genomic clones hybridized in this study (APPENDIX). As expected, each genomic clone and the gene (or genes) included within it hybridized to the same chromosomal site in most cases. However, in four exceptions (*sd*, *Hsp22-26*, *Hsp23-27*, and *tra*) a different localization was observed. These apparent inconsistencies can be resolved by taking into account that in each case the genes are localized at one end of the genomic clone and by assuming that the genomic clone contains a fixed inversion breakpoint. In this case, it seems reasonable to expect a single signal caused by the hybridization of the major portion of the genomic clone instead of the two signals usually seen when a breakpoint is present.

Physical map of the *D. repleta* X chromosome: The localization of the 74 clones from the *D. melanogaster* X chromosome mapped in *D. repleta* is given in the APPENDIX and shown in Figure 2 The euchromatic portion of the *D. melanogaster* X chromosome is ~21.8 Mb long (ADAMS *et al.* 2000) and was divided by Bridges into sections 1–20 (LEFEVRE 1976). Our markers come from all sections of the *D. melanogaster* X chromosome (2–6 markers per section with an average of 3.7 markers) with an average density of 1 marker/295 kb.

All the clones but two hybridized to the X chromosome of *D. repleta*, as expected according to the accepted chromosomal homologies (Figure 1). The two exceptional clones (*Lsp1alpha* and 174F6) likely represent transposition events, which are discussed below. In addition, one gene clone, *CG1716*, gave two hybridization signals in two different *D. repleta* chromosomes: X(F3g) and 4(C3c-d). This gene shows significant sequence homology with two other *D. melanogaster* genes (BERKELEY *DROSOPHILA* GENOME PROJECT 2001): *ash1* localized in 3L(76B9) and *CG4976* localized in 3R(98B2). We interpret the signal in the *D. repleta* X chromosome as pointing to the orthologous gene of *CG1716* in this species whereas the signal in the *D. repleta* chromosome 4 can be tentatively attributed to the orthologous gene of *ash1*.

The physical map of the *D. repleta* X chromosome contains 81 markers (APPENDIX and Figure 2). This includes the 72 markers mapped in this study and a few additional markers mapped previously by our group (RANZ *et al.* 1997, 1999) or other authors (NAVEIRA *et al.* 1986; KOKOZA *et al.* 1992; H. NAVEIRA, personal communication). The genome size of the *repleta* group species is ~220 Mb with 69% (~150 Mb) of single-copy DNA (SCHULZE and LEE 1986). Thus, the euchromatic portion of the *D. repleta* X chromosome, which represents 18% of the total (WASSERMAN 1992), must contain ~27 Mb of DNA and the average marker density is 1 marker per 333 kb. Inspection of Figure 2, however, reveals that the markers are far from being distributed in a uniform manner along this chromosome. If the chromosome is divided in four equal-length quarters, the number of markers in each quarter differs significantly from the random expectation ($G = 21.20$; d.f. = 3, $P < 0.001$) and suggests that gene density varies up to six times between the most distal and most proximal quarters.

Physical map of the *D. repleta* chromosome 4: The localization of the 71 clones from chromosomal arm 3L of *D. melanogaster* successfully hybridized to the chromosomes of *D. repleta* is given in the APPENDIX and shown in Figure 3. The euchromatic portion of chromosomal arm 3L is ~24.4 Mb long in *D. melanogaster* (ADAMS *et al.* 2000) and is composed of sections 60–80 of the cytological map drawn by Bridges (LEFEVRE 1976). We have mapped 1–6 markers per section (average 3.5 markers) with an average density of 1 marker per 344 kb.

All 71 clones hybridized to chromosome 4 of *D. repleta* (APPENDIX), which is homologous to chromosomal arm 3L of *D. melanogaster* (Figure 1). Thus, after including 8 markers from our previous work (RANZ *et al.* 1997) and those of other authors (NAVEIRA *et al.* 1986; LAAYOUNI *et al.* 2000), the physical map of *D. repleta* chromosome 4 bears 79 markers (APPENDIX and Figure 3). The size of the euchromatic portion of chromosome 4 is ~27 Mb and the average density is 1 marker per 342 kb, both values similar to those for chromosome X. In contrast to the previous results of the X chromosome, however, the markers are distributed uniformly along chromosome 4 with similar numbers in the four quarters ($G = 1.72$, d.f. = 3, $P > 0.05$).

DISCUSSION

Exceptions to the chromosomal homologies and rate of gene transposition: The ancestral karyotype of the genus Drosophila consisted of five acrocentric chromosomes and a dot (Muller's elements A–F). Our results are in good agreement with an extensive conserved syn-

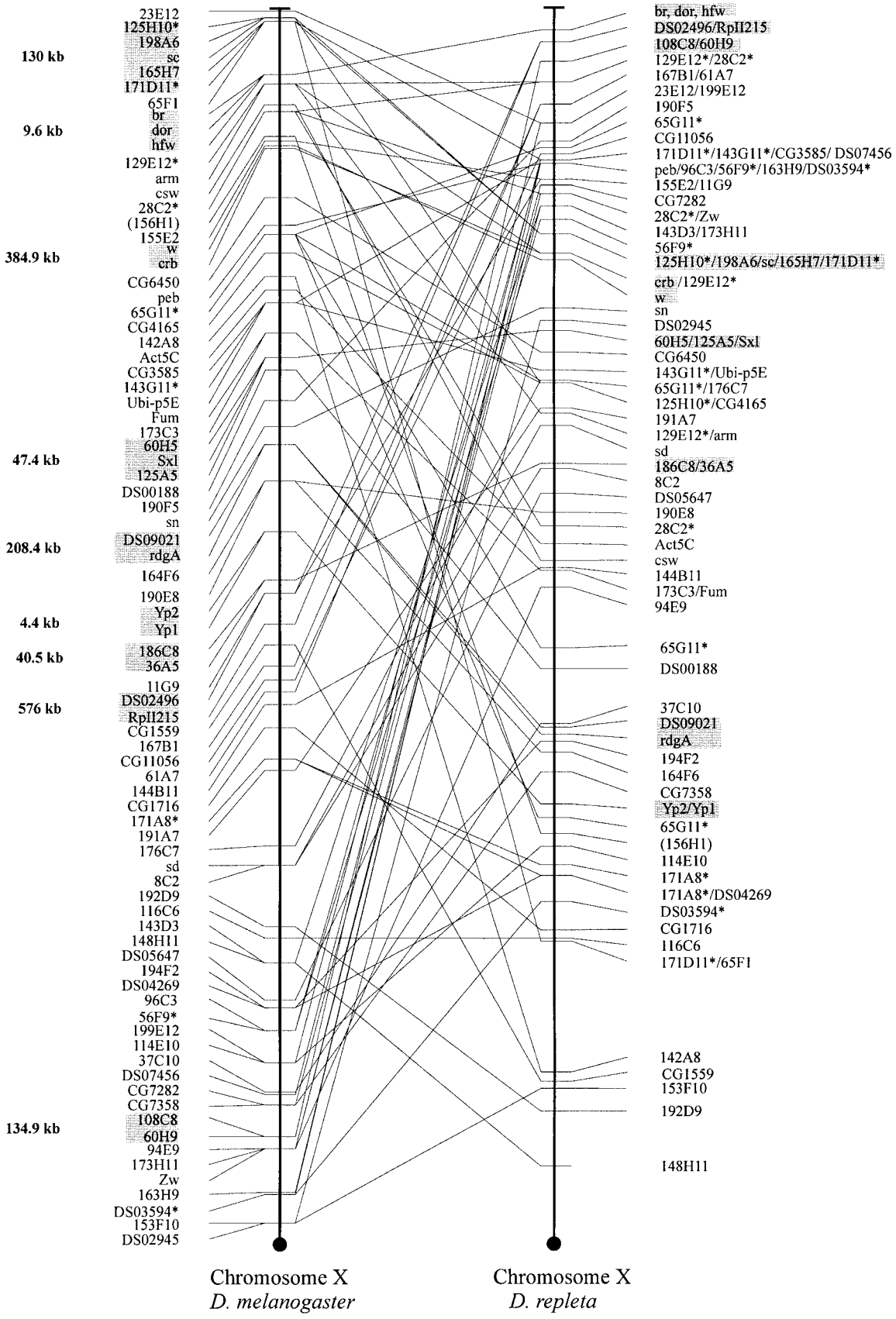

FIGURE 2.—Comparison of the molecular organization of Muller's element A (chromosome X) between *D. melanogaster* and *D. repleta*. Connecting lines match the cytological position of orthologous markers. Shaded rectangles show conserved segments with two or more consecutive markers. The estimated size of each conserved segment is given on the leftmost column. The asterisk (*) indicates those clones yielding more than one hybridization signal. The names of those clones in parentheses are incorrect (these clones have probably been mislabeled during the clone distribution process).

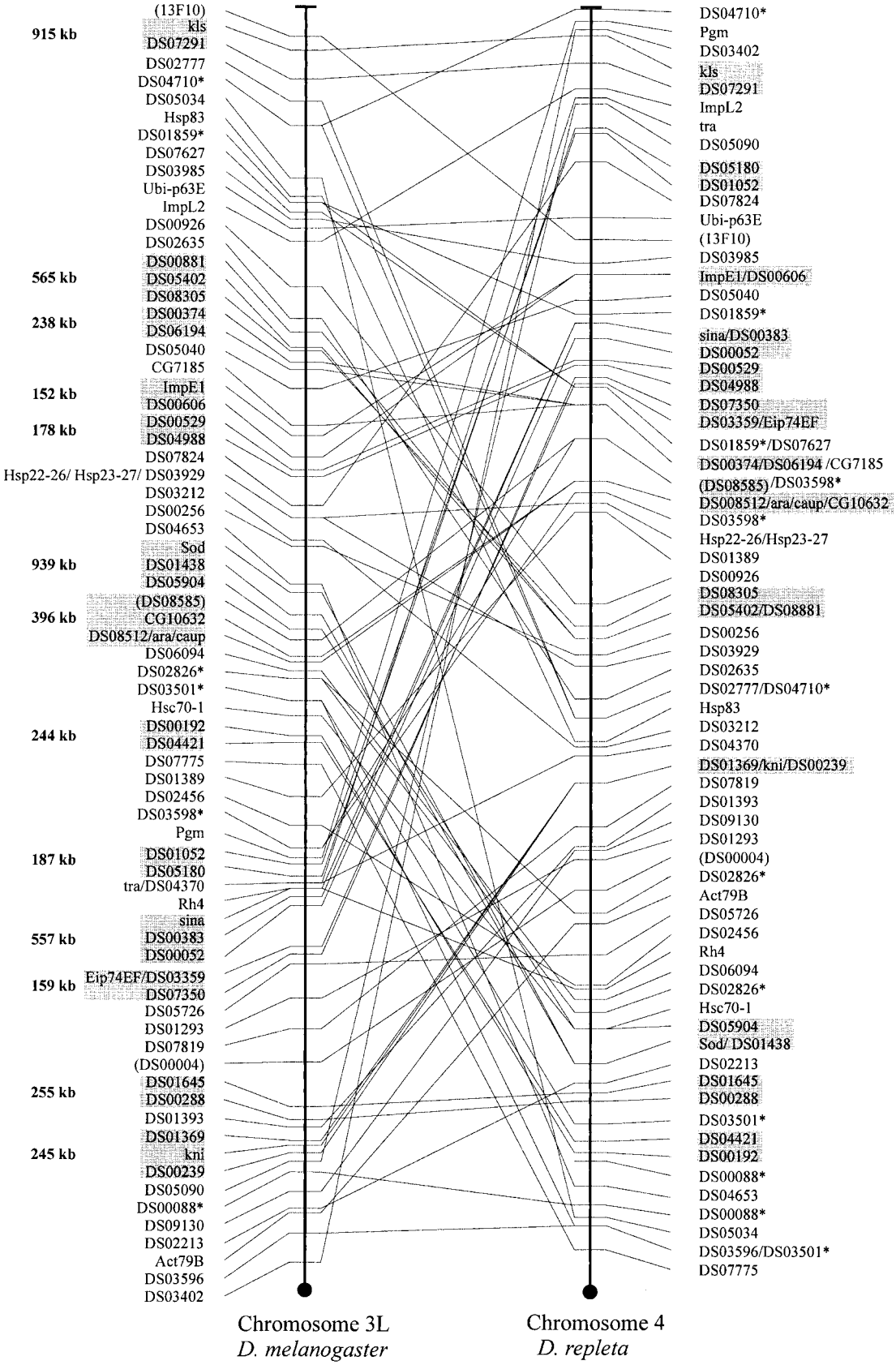

FIGURE 3.—Comparison of the molecular organization of Muller's element D between *D. melanogaster* (chromosomal arm 3L) and *D. repleta* (chromosome 4). For meaning of the symbols, see legend of Figure 2.

teny of Muller's elements A and D during the evolution of the *D. melanogaster* and *D. repleta* lineages. Therefore, and with the exception of 2 out of 145 clones, the established chromosomal homologies between chromosome X of *D. melanogaster* and *D. repleta* and between chromosomal arm 3L of *D. melanogaster* and chromosome 4 of *D. repleta* (Figure 1) are firmly corroborated. Our results also indicate that no exchange of information occurred via pericentric inversions after the centric fusion between Muller's elements D and E that gave rise to the metacentric chromosome 3 in the *D. melanogaster* lineage. Mammals also show an extensive conserved synteny of chromosome X, even though translocations have often rearranged the genome of mammalian species (Ohno 1967; Lander *et al.* 2001). However, the conservation of the X chromosome in mammals and in the Drosophila genus likely results from different mechanisms. In the case of mammals, there is a need to keep the level of expression of the X-linked genes adjusted to one single copy due to the dosage compensation mechanism (Hartl and Lozovskaya 1994; Graves 1996). In Drosophila, without discarding adjustments on gene dosage, the main reason is probably the reported lack of interchromosomal rearrangements, which holds for both the X chromosome and the autosomes (Powell 1997).

Because of this absence of interchromosomal rearrangements in Drosophila, the two exceptional clones that fail to obey the synteny conservation likely indicate transposition events.

i. The gene *Lsp1alpha* is located on the X chromosome (element A) of *D. melanogaster* but maps to chromosome 2 (element E) of *D. repleta*. This gene is also localized in element E in eight different species belonging to the Sophophora and Drosophila subgenera (Brock and Roberts 1983) and it has been suggested that it recently transposed onto the X chromosome in the species belonging to the *melanogaster* subgroup (Smith *et al.* 1981). The *D. buzzatii Lsp1* genes are being cloned and sequenced in our laboratory to test this hypothesis (J. González, F. Casals and A. Ruiz, unpublished results).
ii. Cosmid clone 174F6 maps to the euchromatin-heterochromatin boundary of the *D. melanogaster* X chromosome (20A-C). However, it hybridized near the centromere (polytene band G5d) of chromosome 4 in *D. repleta*. This cosmid clone contains the *suppressor of forked* [*su(f)*] gene (Madueño *et al.* 1995). Analysis of a 33-kb chromosomal walk around the *su(f)* locus in *D. melanogaster* revealed that most of this interval consists of repetitive sequences. In fact the *su(f)* gene is flanked by a 1.5-kb direct repeat sequence (Tudor *et al.* 1996). Sequences homologous to the 1.5-kb repeats are found in the euchromatin-heterochromatin boundary of chromosome arms 2L, 2R, and 3L of *D. melanogaster*. Therefore, ectopic exchange events involving some homology between donor and target site, which are a possible mechanism of gene transposition in the Drosophila genome (Yi and Charlesworth 2000), could explain the case of cosmid clone 174F6.

A crude estimate of the rate of gene transposition in the Drosophila genus can be produced by combining the results of the present work with results previously obtained for chromosomal elements B (González *et al.* 2000) and E (Ranz *et al.* 2001). In these latter works no cases of gene transposition were detected. Therefore we have observed two possible transposition events out of a total of 328 clones hybridized to the *D. repleta* chromosomes (Table 1). This amounts to a rate of 4.9×10^{-5} transpositions/gene/myr, which is quite low. This rate, however, does not include tandemly repeated genes such as histone or rRNA genes, which often show transposition (Alonso and Berendes 1975; Fitch *et al.* 1990). It also does not include intrachromosomal transpositions. Given the differentiation undergone by the banding pattern and morphology of the polytene chromosomes of so distantly related species as *D. repleta* and *D. melanogaster*, the only transpositions that we can safely detect with our mapping procedure are those taking place between different chromosomal elements. Transpositions within the same chromosomal element are probably overlooked, although they do exist. For instance, the *seven in absentia* (*sina*) gene is nested within an intron of the *Rh4* opsin gene in chromosomal arm 3L of *D. melanogaster* (Montell *et al.* 1987). However, in *D. virilis* (Neufeld *et al.* 1991) and *D. repleta* (this work) the two genes are located at distant sites of the homologous element (chromosome 3 in *D. virilis* and chromosome 4 in *D. repleta*). To explain the different structural arrangement of these two genes between *D. melanogaster* and *D. virilis*, Neufeld *et al.* (1991) proposed a retrotransposition event of the *Rh4* gene in the lineage leading to *D. virilis*. Our results support their interpretation because two P1 phages (DS00383 and DS00052) from the *sina/Rh4* region in *D. melanogaster* map near *sina* but far from *Rh4* in *D. repleta*. In addition, if a single transposition event was involved, our result indicates that it took place after the separation of the *D. melanogaster* and *D. virilis* ancestral lineages but before the divergence between the *D. virilis* and *D. repleta* lineages (~30 myr ago). Further work with phylogenetically closer species, whose polytene chromosomes are more easily compared, is required to obtain more accurate estimates of the rate of gene transposition.

Comparative mapping and rates of fixation of paracentric inversions: Our fairly dense physical maps of *D. repleta* chromosomes X and 4 allow a detailed comparison of their gene arrangements with those of the homologous *D. melanogaster* elements X and 3L (Figures 2 and 3). In both elements, a considerable reshuffling of gene order extends from telomere to centromere. The rank

TABLE 3

Rates of chromosomal evolution since the divergence between *D. melanogaster* and *D. repleta*

Muller's chromosomal element	A	B	D	E	A–F
Size of euchromatic portion (Mb) in *D. melanogaster*	21.8	23.0	24.4	28.0	120
Estimated no. of breakpoints ±SD	236 ± 33	59 ± 25	113 ± 16	228 ± 28	785
Breakpoint density (Mb^{-1}) ±SD	10.83 ± 1.56	2.57 ± 1.10	4.63 ± 0.66	8.14 ± 1.00	6.54
Predicted length of conserved segments (kb)	92.0	383.3	214.0	122.3	200.5
No. of inversions fixed ±SD	118 ± 17	30 ± 13	56 ± 8	114 ± 14	393
Evolution rate (disruptions/Mb/myr)	0.087	0.021	0.037	0.066	0.053

Data from González *et al.* (2000), Ranz *et al.* (2001), and this work.

order correlation is in both cases nonsignificant (for chromosome X, Spearman's R = 0.062, $P > 0.05$, six ties; for chromosome 4, Spearman's R = 0.184, $P > 0.05$, two ties), indicating that gene order is effectively randomized. This profound rearrangement found for Muller's elements A and D can be attributed mainly to the fixation of paracentric inversions if we consider that transposition rates are low (see above) and that paracentric inversions are the prevailing chromosomal rearrangement in Drosophila both as intraspecific polymorphisms and as interspecific fixed differences (Hartl and Lozovskaya 1994; Powell 1997; Ranz *et al.* 2001). Using the maximum-likelihood method devised by Ranz *et al.* (1997) we have estimated the number of inversions fixed between *D. repleta* and *D. melanogaster* since their most recent common ancestor (Figure 1). An estimate (±SD) of 118 ± 17 for Muller's element A and 56 ± 8 for Muller's element D were obtained (Table 3). The coefficient of variation (CV) of both estimates is reasonably low (14%) and comparable to the most detailed comparative maps carried out in Drosophila (Ranz *et al.* 2001). Obviously, the accuracy with which the rates of chromosomal evolution are estimated increases with the number of markers used in comparative mapping (Schoen 2000). However, computer simulations made with the method of Ranz *et al.* (1997) show that the CV is not a lineal function of the number of markers but follows a negative exponential function (D. Schoen, personal communication). This implies that a decrease of the CV below the actual values would require a disproportionate increase in the number of markers.

The evolution rates for chromosomal elements A and D can be compared with those for elements B and E, which have been previously estimated using the same pair of species (González *et al.* 2000; Ranz *et al.* 2001). Because the different chromosomal elements vary in size, to make the data comparable, we have calculated the density of breakpoints per megabase by dividing the number of breakpoints by the size of each element in megabases in *D. melanogaster* (Table 3). Breakpoint density varies up to four times among chromosomal elements and the differences are statistically significant. The breakpoint density for element A (X chromosome) is the highest (10.83) but there are differences between the three analyzed autosomes as well. Element E exhibits the highest density (8.14) whereas element B shows the lowest (2.57) and element D is intermediate (4.63). The weighted average for the whole genome (the euchromatic portion of the six chromosomal elements A–F) is 6.54 breakpoints/Mb, which allows us to infer that 393 paracentric inversions have become fixed in the whole genome between *D. melanogaster* and *D. repleta* (Table 3). Taking 62 myr as the divergence time between the two subgenera, we obtain conservative estimates for the rate of disruptions per megabase per million years. These estimates range from 0.021 for element B up to 0.087 for element A with a weighted average of 0.053 for the whole genome (Table 3).

These results agree fairly well with the scarce reliable estimates previously reported in other Drosophila species. Segarra *et al.* (1995) compared the X chromosome between *D. melanogaster* and *D. pseudoobscura* and estimated that 0.086 disruptions/Mb/myr have occurred since the divergence of these two lineages. Likewise, Vieira *et al.* (1997a,b) compared the gene order of three different chromosomes among *D. virilis*, *D. montana*, and *D. novamexicana*, three species of the *virilis* group of subgenus Drosophila. Their rates (taking *D. melanogaster* as the reference species for chromosome sizes as before) were 0.036–0.056 disruptions/Mb/myr for chromosome X, 0.032 for chromosome 2 (Muller's element E), and 0.009–0.014 for chromosome 3 (Muller's element D). It is remarkable that the ranking order between chromosomal elements in these studies (A > E > D) is the same that we have observed (Table 3), which suggests a genus-wide pattern regardless of the phylogenetic distance of the species compared.

In addition, our results support the previous finding that the rate of genome rearrangement in Drosophila is about two orders of magnitude higher than that in mammals and several times higher than that in the most dynamic plant lineages (Ranz *et al.* 2001). This conclusion was drawn from the comparative analysis of the Muller's element E between *D. melanogaster* and *D. repleta*, which represents the ~23% of the euchromatic fraction of the *D. melanogaster* genome. Now, with comparative data in *D. repleta* of >60% of the *D. melanogaster* genome, the same conclusion still holds. Most current

TABLE 4

Basic features of the chromosomal elements of *D. melanogaster*

Chromosomal arm	Size (cM/Mb)[a]	Recombination rate (cM/Mb)	Genes[b]		Transposable elements[c]		Microsatellites[d]	
			Number	Density	Number	Density	Number	Density
X	73.1/21.8	3.35	2,314	106.1	175	8.03	13,658	626.5
2L	55/23.0	2.39	2,378	103.4	213	9.26	6,012	261.4
2R	55/21.4	2.57	2,616	122.2	212	9.91	6,264	292.7
3L	47/24.4	1.93	2,583	105.9	182	7.46	7,157	293.3
3R	63.9/28.0	2.28	3,357	119.9	183	6.54	8,666	309.5
4	3/1.2	2.5	83	69.2	42	35.00		

[a] Lindsley and Zimm (1992) and Adams *et al.* (2000)
[b] S. Misra (Berkeley *Drosophila* Genome Project), personal communication.
[c] Rizzon *et al.* (2002) and C. Rizzon, personal communication.
[d] Katti *et al.* (2001).

comparative maps of mammals (and also plants) have a relatively poor resolution. Consequently, as the number of orthologous markers mapped increase, it is likely that more rearrangements (*e.g.*, paracentric inversions) will be discovered in some lineages and the evolution rates in these lineages rise accordingly (Sun *et al.* 1999; Müller *et al.* 2000; Puttagunta *et al.* 2000; Frönicke and Wienberg 2001). In our view, however, such increase will not equalize the disparate evolution rates that we have observed. The plausible reasons for the faster chromosomal evolution in the genus Drosophila have been discussed elsewhere (Ranz *et al.* 2001).

What factors can account for the remarkable variation in evolution rate observed between the chromosomal elements of Drosophila? Factors affecting the rate of chromosomal evolution can be classed in two groups: mutational and selective. A higher rate of inversion fixation would be expected if mutation rate were higher (other things being equal). In Drosophila, transposable elements (TEs) have been implicated in the origin of natural inversions, which can originate through ectopic recombination between TE copies located in opposite orientation in different sites of the same chromosome (Montgomery *et al.* 1991; Lim and Simmons 1994; Andolfatto *et al.* 1999; Cáceres *et al.* 1999a, 2001; Mathiopoulos *et al.* 1999). Thus, a higher mutation rate could be due to a higher proportion of repetitive DNA or to a higher recombination rate. If we consider the recently sequenced genome of *D. melanogaster* (Table 4), it would appear that the X chromosome, which in our study showed the highest breakpoint density, does not show a TE density higher than that of the autosomes (Rizzon *et al.* 2002). On the other hand, chromosome X does possess a microsatellite density that is at least twice that of any of the autosomes (Katti *et al.* 2001), a fact that likely reflects the presence of several repeated sequences that are exclusive of the X chromosome euchromatin or more abundant in the X chromosome than in the autosomes (Huijser *et al.* 1987; Pardue *et al.* 1987; Waring and Pollack 1987; Lowenhaupt *et al.* 1989; Dibartolomeis *et al.* 1992; Bachtrog *et al.* 1999). Moreover, the microsatellite density of the autosomes (Table 4) parallels their evolution rates (Table 3). This is an intriguing observation because microsatellite sequences can generate unstable secondary structures (Mitas 1997; Moore *et al.* 1999) that could be involved in the origin of chromosome rearrangements (Pletcher *et al.* 2000; Puttagunta *et al.* 2000). So far, however, no evidence for the implication of microsatellites in the origin of Drosophila inversions has been found. Obviously, more data are needed on the distribution of TEs and repeated sequences among chromosomal elements in other Drosophila species apart from *D. melanogaster*.

The second factor that might be affecting inversion production is recombination rate. If we assume that ectopic recombination is correlated with regular meiotic recombination (Montgomery *et al.* 1991), then meiotic recombination rates in *D. melanogaster* can be considered in search of a pattern. Chromosome X exhibits a higher average recombination rate (3.35 cM/Mb) than the autosomes (Table 4) in good agreement with its faster chromosomal evolution. However, the average recombination rate of chromosomal arm 2L, which shows the slowest evolution rate, is comparable (or superior) to that of both arms of chromosome 3 (Table 4). Therefore, no consistent effect of recombination on evolution rate is apparent. On the other hand, it is clear that recombination rates vary between Drosophila species (True *et al.* 1996; Cáceres *et al.* 1999b) and the *D. melanogaster* rates may not have a genus-wide validity.

Another group of factors comprises those selective causes affecting the probability of fixation of inversions. For instance, Charlesworth *et al.* (1987) showed that the X chromosome should evolve faster than the autosomes due to a higher fixation probability of underdominant and favorable partial or fully recessive rearrangements. Also, when the chromosomal rearrangements

have an antagonistic effect in the two sexes, they will invade the population under a wider range of conditions if they are X linked than if they occur in the autosomes (Rice 1984). These predictions might help to explain the fast evolution rate of the X chromosome but would not explain the rate variation between the autosomes. A higher evolution rate could also be due to less functional constraints, as would be expected in regions with low gene density (Lander *et al.* 2001). Fixed inversions are more likely to have their breakpoints between genes (as found by Cirera *et al.* 1995 and Cáceres *et al.* 1999a) than within transcriptions units (as in Schneuwly *et al.* 1987). In the latter case, an inversion would probably have a strong deleterious effect and would be quickly eliminated by natural selection before fixation. The average density in Drosophila is one gene per 9 kb but there is substantial variation in gene density throughout the genome (Adams *et al.* 2000). Nevertheless, the average gene density (as inferred from release 2 of the *D. melanogaster* genome sequence; Adams *et al.* 2000) seems to be comparable for elements X, 2L, and 3L and only slightly higher for elements 2R and 3R (Table 4). Thus, no systematic correlation is apparent between gene density and evolution rate in Drosophila. However, we have to take into account that there can be local differences in gene density within chromosomal elements. Jabbari and Bernardi (2000) pointed out that the gene concentration in GC-rich regions is sevenfold higher than that in GC-poor regions in the Drosophila genome and our results suggest comparable density differences within the *D. repleta* X chromosome. Chromosomal arms rich in gene-poor intervals might have more fixed breakpoints than arms with little or no variation in gene density.

Chromosomal inversions may have diverse effects at the genetic and phenotypic level, which will affect their probability of fixation in a complex manner. For instance, in heterokaryotypes, inversions reduce recombination rate in the inverted chromosomal segment (Navarro *et al.* 1997) but may increase it in the nonhomologous chromosomes (Lucchesi and Suzuki 1968). Accordingly, the fate of an inversion is considered to depend strongly on the epistatic combinations of alleles caught by the inversion at the moment of its appearance (Charlesworth and Charlesworth 1973; Charlesworth 1974) and the species recombination rate (Cáceres *et al.* 1999b). Finally, in some cases (*e.g.*, the sex ratio arrangement of chromosome X), inversions may be associated with meiotic drive alleles and be preferentially transmitted to the offspring (Ashburner 1989). Given these manifold effects of inversions, it seems improbable that a single factor explains the variation in evolution rate among Drosophila chromosomal elements and we must cautiously conclude that several causes, some of them discussed above, contribute to this variation.

Conserved chromosomal segments and functional constraints: The chromosomes of *D. repleta* can be regarded as a mosaic of relatively small segments homologous to those in *D. melanogaster* chromosomes. Our estimates of the number of inversions fixed in elements A and D allow us to predict that the average size of such segments is 92 kb for chromosome X and 214 kb for chromosome 4 (Table 3). The comparison of the physical maps of *D. melanogaster* and *D. repleta* led us to the identification of 9 and 13 conserved segments with two or more consecutive markers in elements A and D, respectively (see Figures 2 and 3). There were also 39 and 29 singletons, segments that contained only a single marker. In chromosome X the size of the 9 segments with two or more consecutive markers ranged from 4.4 to 576 kb with an average length of 170.7 kb. Likewise, in chromosome 4 the size of the 13 conserved segments ranged from 152 to 939 kb with an average length of 386.9 kb. In both cases, the average length of the observed conserved segments is bigger than the predicted average size (Table 3). This is expected because there is a discovery bias that favors big conserved segments at the initial stages of comparative mapping and also because only conserved segments delimited by two or more markers have been considered to estimate the average length of observed segments (Nadeau and Sankoff 1998b).

Blocks of genes that are conserved during long periods of time may represent gene combinations that interact functionally and are therefore maintained together by natural selection, the so-called "functional constraints" hypothesis (Maier *et al.* 1993; Randazzo *et al.* 1993; Wright 1996). However, because all genomes are phylogenetically related, colinear groups of genes may also reflect the fixation of a limited number of genomic rearrangements with random breakpoints since both species diverged, the "random breakage" (RB) hypothesis (Nadeau and Taylor 1984; Nadeau and Sankoff 1998a). Previous comparative mapping results in Drosophila (González *et al.* 2000; Ranz *et al.* 2001) and other organisms (Nadeau and Sankoff 1998a; Huynen *et al.* 2001; Lander *et al.* 2001) have found little evidence for functional constraints. The RB hypothesis can be tested comparing the observed length distribution of conserved segments with that predicted under the RB hypothesis, which will approximate a negative exponential distribution (Figure 4). It can be seen that, in both chromosomal elements, the empirical distribution fits in general inside the theoretical distribution as expected because only a subset of all conserved segments has been detected. Only one segment in each chromosome seems to depart significantly from the expectations (Figure 4). The size and gene content of these segments should be further investigated.

We can look at the functional constraints hypothesis from a different perspective. There are a few examples of gene complexes in *D. melanogaster* chromosomes X and 3L whose members show a coregulated expression. We can ask whether or not such complexes are con-

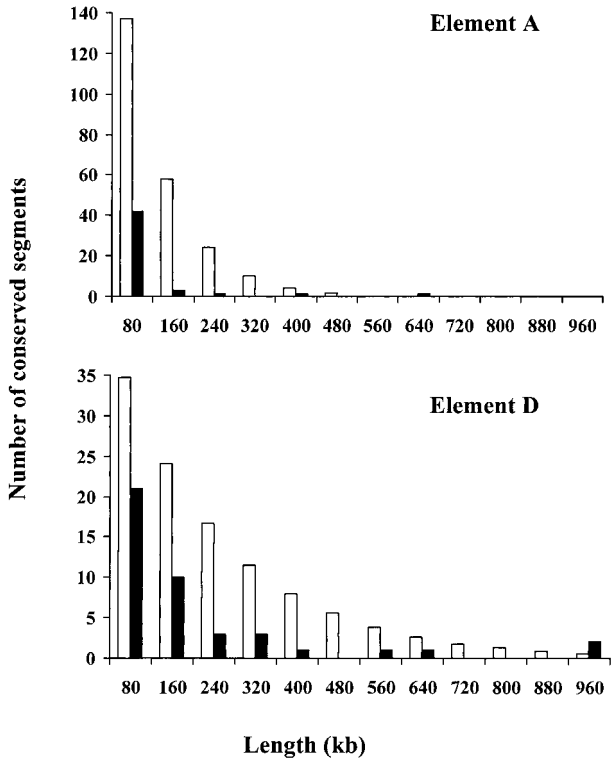

FIGURE 4.—Expected (□) and observed (■) distribution of the length of conserved segments under the random breakage hypothesis (NADEAU and TAYLOR 1984; NADEAU and SANKOFF 1998b). The empirical distribution fits in general inside the theoretical distribution as expected because only a subset of all conserved segments has been detected (see text for details).

served in *D. repleta*. The *achaete-scute* complex (AS-C) has been studied extensively (reviewed in MODOLELL and CAMPUZANO 1998). It spans ~90 kb of the X chromosome where only six transcription units are separated by very large stretches of nontranscribed DNA. This DNA contains many *cis*-regulatory sequences that coregulate the *achaete* and *scute* (but not the *lethal-of-scute* and *asense*) genes of the complex. The molecular organization of the 210-kb *D. melanogaster* segment delimited by the markers 125H10-65F1 has been studied in *D. repleta*. This 210-kb segment contains, among others, the genes of the AS-C (see APPENDIX). When the physical maps of this region are compared, only a segment of ~130 kb, which includes the genes *achaete* and *scute*, has been conserved. This gene complex is also conserved in *D. virilis* (BEAMONTE 1990). Thus, the molecular organization of this gene complex seems to have been preserved during the 80–124 myr of divergence of these species. Two gene complexes of chromosome 3L also appear to show coregulated expression. The genes *araucan* (*ara*) and *caupolican* (*caup*), two members of the Iroquois complex, have similar patterns of expression and apparently share *cis*-regulatory sequences (GÓMEZ-SKARMETA *et al.* 1996). They are closely linked in *D. melanogaster* (comprising a genomic segment of ~40 kb) and also in *D. repleta* (as shown by the coincident hybridization sites of *caup*, *ara*, and DS08512). Finally, *knirps* (*kni*) and *knirps-related* (*knrl*) are two neighboring and functionally equivalent genes mapping to a region of 100 kb (LUNDE *et al.* 1998). They are affected by a *cis*-acting regulatory sequence (*ri*) lying immediately upstream of the *kni* transcription unit. A chromosomal segment of 245 kb around the *kni* locus (comprising *kni*, DS01369, and DS00239) is conserved in *D. repleta*, suggesting that both genes and their regulatory sequences have not been disrupted. In summary, the conservation of these three small gene complexes suggest that natural selection may play a role in some (perhaps exceptional) cases to keep together functionally related Drosophila genes.

Overall our results are in agreement with a modular organization of the Drosophila genome (RANZ *et al.* 2001). Thus, the genome of Drosophila can be seen as a mosaic of independent modules that can change their localization within the euchromatin without loss of function. Usually these modules change their localization within the chromosomal arm and only occasionally between chromosomal arms. Each module may consist of a gene plus its regulatory sequences (as proposed by RANZ *et al.* 2001) or perhaps a small group of nearby genes. If Drosophila euchromatin possess expression domains organized by insulators or boundary elements (GERASIMOVA *et al.* 2000; MONGELARD and CORCES 2001), this undoubtedly will influence the molecular consequences of inversion breakpoints. We can speculate that breaks taking place within trancriptionally independent domains will have more disturbing consequences than those occurring between domains. The rough agreement between the size of these expression domains (GERASIMOVA *et al.* 2000) and the average size of the conserved segments (Table 3) is certainly intriguing and deserves more work. Recent comparisons of yeast genomes show the prevalence of small inversions in gene order evolution between Saccharomyces and some Candida species (LLORENTE *et al.* 2000; SEOIGHE *et al.* 2000). A similar result was found when comparing zebrafish and human genomes (POSTLETHWAIT *et al.* 2000), supporting the hypothesis that inversions have been a more frequent force in the shaping of vertebrate karyotypes than translocations. Altogether these results suggest a major role for inversions in the genome shuffling process (HUYNEN *et al.* 2001).

We thank all the authors who sent us clones and/or information: A. Bidwai (West Virginia University); H. Biessman (University of California, Irvine); O. Cabré (Universitat Autònoma de Barcelona); A. Chovnik (University of Connecticut, Storrs); C. Ferraz (Centre National de la Recherche Scientifique); J. W. Fristrom (University of California, Berkeley); R. de Frutos (Universidad de Valencia); C. Kraemer (Universität Mainz); E. Madueño, J. Modolell, and S. Campuzano (Centro de Biología Molecular Severo Ochoa, CSIC, Madrid); D. G. McEwen (University of North Carolina); H. Naveira (Universidade da Coruña); K. O'Hare (Imperial College Science Technology, London); N. Perrimon (Harvard Medical School, HHMI, Boston);

and L. Sánchez (Centro de Investigaciones Biológicas, CSIC, Madrid). We are grateful to F. Casals for his help with the cloning of *sina* and *Rh*, to S. Misra (Berkeley *Drosophila* Genome Project) for the analysis of the GadFly database, and to D. Schoen for his computer simulations of the inversion fixation process. The helpful comments of E. Betrán, J. Modolell, C. Segarra, D. Shoen, A. Villasante, and two anonymous referees contributed to improve previous versions of the manuscript. Work was supported by grant PB98-0900-C02-01 from the Dirección General de Enseñanza Superior e Investigación Científica (Ministerio de Educación y Cultura; Spain) awarded to A.R. and a doctoral FI fellowship from the Universitat Autònoma de Barcelona awarded to J.G.

LITERATURE CITED

Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne *et al.*, 2000 The genome sequence of *Drosophila melanogaster*. Science **287:** 2185–2195.

Alonso, C., and H. D. Berendes, 1975 The location of 5S (ribosomal) RNA genes in *Drosophila hydei*. Chromosoma **51:** 347–356.

Andolfatto, P., J. D. Wall and M. Kreitman, 1999 Unusual haplotype structure at the proximal breakpoint of *In(2L)t* in a natural population of *Drosophila melanogaster*. Genetics **153:** 1297–1311.

Andres, A. J., J. C. Fletcher, F. D. Karim and C. S. Thummel, 1993 Molecular analysis of the initiation of insect metamorphosis: a comparative study of Drosophila ecdysteroid-regulated transcription. Dev. Biol. **160:** 388–404.

Ashburner, M., 1989 *Drosophila: A Laboratory Handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Bachtrog, D., S. Weiss, B. Zangerl, G. Brem and C. Schlötterer, 1999 Distribution of dinucleotide microsatellites in the *Drosophila melanogaster* genome. Mol. Biol. Evol. **16:** 602–610.

Barnett, T., C. Pachl, J. P. Gergen and P. C. Wensink, 1980 The isolation and characterization of Drosophila yolk protein genes. Cell **21:** 729–738.

Beamonte, D., 1990 Búsqueda y caracterización de genes homólogos a los del complejo *achaete-scute* de *Drosophila melanogaster*. Ph.D. Thesis, Universidad Autónoma de Madrid, Spain.

Berkeley *Drosophila* Genome Project, 2001 The Berkeley *Drosophila* Genome Project database (available from http://www.fruitfly.org).

Beverly, S. M., and A. C. Wilson, 1984 Molecular evolution in Drosophila and higher Dipterans. II. A time scale for fly evolution. J. Mol. Evol. **21:** 1–13.

Brock, H. W., and D. B. Roberts, 1983 Location of the *LSP-1* genes in Drosophila species by *in situ* hybridization. Genetics **103:** 75–92.

Burt, D. W., C. Bruley, I. C. Dunn, C. T. Jones, A. Ramage *et al.*, 1999 The dynamics of chromosome evolution in birds and mammals. Nature **402:** 411–413.

Cáceres, M., J. M. Ranz, A. Barbadilla, M. Long and A. Ruiz, 1999a A transposable element mediated the generation of a Drosophila widespread chromosomal inversion. Science **285:** 415–418.

Cáceres, M., A. Barbadilla and A. Ruiz, 1999b Recombination rate predicts inversion size in Diptera. Genetics **153:** 251–259.

Cáceres, M., M. Puig and A. Ruiz, 2001 Molecular characterization of two natural hotspots in the *Drosophila buzzatii* genome induced by transposon insertions. Genome Res. **11:** 1353–1364.

Campbell, S. D., A. Duttaroy, A. L. Katzen and A. Chovnick, 1991 Cloning and characterization of the *scalloped* region of *Drosophila melanogaster*. Genetics **127:** 367–380.

Charlesworth, B., 1974 Inversion polymorphism in a two locus genetic system. Genet. Res. **23:** 259–280.

Charlesworth, B., and D. Charlesworth, 1973 Selection of new inversions in a multilocus genetic system. Genet. Res. **21:** 167–183.

Charlesworth, B., J. A. Coyne and N. H. Barton, 1987 The relative rates of evolution of sex chromosomes and autosomes. Am. Nat. **130:** 113–146.

Cirera, S., J. M. Martín-Campos, C. Segarra and M. Aguadé, 1995 Molecular characterization of the breakpoints of an inversion fixed between *Drosophila melanogaster* and *D. subobscura*. Genetics **139:** 321–326.

Córces, V., R. Holmgrem, R. Freund, R. Morimoto and M. Meselson, 1980 Four heat shock proteins of *Drosophila melanogaster* coded within a 12-kilobase region in chromosome subdivision 67B. Proc. Natl. Acad. Sci. USA **9:** 5390–5393.

DiBartolomeis, S. M., K. D. Tartof and F. R. Jackson, 1992 A superfamily of Drosophila satellite related (SR) DNA repeats restricted to the X chromosome euchromatin. Nucleic Acids Res. **20:** 1113–1116.

Ehrlich, J., D. Sankoff and J. H. Nadeau, 1997 Synteny conservation and chromosome rearrangements during mammalian evolution. Genetics **147:** 289–296.

Fitch, D. H. A., L. D. Strausbaugh and V. Barrett, 1990 On the origins of tandemly repeated genes: Does histone gene copy number in Drosophila reflect chromosomal location? Chromosoma **99:** 118–124.

Frönicke, L., and J. Wienberg, 2001 Comparative chromosome painting defines the high rate of karyotype changes between pigs and bovids. Mamm. Genome **12:** 442–449.

Gale, M. D., and K. M. Devos, 1998 Plant comparative genetics after 10 years. Science **282:** 656–659.

Ganguly, R., N. Ganguly and J. E. Manning, 1985 Isolation and characterization of the glucose-6-phosphate dehydrogenase gene of *Drosophila melanogaster*. Gene **35:** 91–101.

Gerasimova, T. I., K. Byrd and V. G. Corces, 2000 A chromatin insulator determines the nuclear localization of DNA. Mol. Cell **6:** 1025–1035.

Gómez-Skarmeta, J. L., R. Díez del Corral, E. de la Calle-Mustienes, D. Ferrés-Marcó and J. Modolell, 1996 *araucan* and *caupolican*, two members of the novel *Iroquois* complex, encode homeoproteins that control proneural and vein-forming genes. Cell **85:** 95–105.

González, J., E. Betrán, M. Ashburner and A. Ruiz, 2000 Molecular organization of the *Drosophila melanogaster Adh* chromosomal region in *D. repleta* and *D. buzzatii*, two distantly related species of the Drosophila subgenus. Chromosome Res. **8:** 375–385.

Gorman, M., A. Franke and B. S. Baker, 1995 Molecular characterization of the male-specific lethal-3 gene and investigations of the regulation of dosage compensation in Drosophila. Development **121:** 463–475.

Graves, J. A. M., 1996 Mammals that break the rules: genetics of marsupials and monotremes. Annu. Rev. Genet. **30:** 233–260.

Hartl, D. L., and E. R. Lozovskaya, 1994 Genome evolution: between the nucleosome and the chromosome, pp. 579–592 in *Molecular Ecology and Evolution: Approaches and Applications*, edited by B. Schierwater, B. Streit, G. P. Wagner and R. DeSalle. Birkhäuser, Basel, Switzerland.

Hartl, D. L., and E. R. Lozovskaya, 1995 *The Drosophila Genome Map: A Practical Guide*. Springer-Verlag, New York.

Hartl, D. L., D. I. Nurminsky, R. W. Jones and E. R. Lozovskaya, 1994 Genome structure and evolution in Drosophila: applications of the framework P1 map. Proc. Natl. Acad. Sci. USA **91:** 6824–6829.

Holmgren, R., V. Córces, R. Morimoto, R. Blackman and M. Meselson, 1981 Sequence homologies in the 5′ regions of four Drosophila heat-shock genes. Proc. Natl. Acad. Sci. USA **6:** 3775–3778.

Hudson, R. R., K. Bailey, D. Skarecky, J. Kwiatowski and F. J. Ayala, 1994 Evidence for positive selection in the superoxide dismutase (*Sod*) region of *Drosophila melanogaster*. Genetics **136:** 1329–1340.

Huijser, P., W. Hennig and R. Dijkhof, 1987 Poly (dC-dA/dG-dT) repeats in the Drosophila genome: A key function for dosage compensation and position effects? Chromosoma **95:** 209–215.

Hurst, L. D., 2001 Sex and the X. Nature **411:** 149–150.

Huynen, M. A., B. Snel and P. Bork, 2001 Inversions and the dynamics of eukaryotic gene order. Trends Genet. **17:** 304–306.

Jabbari, K., and G. Bernardi, 2000 The distribution of genes in the Drosophila genome. Gene **247:** 287–292.

Katti, M. V., P. K. Ranjekar and V. S. Gupta, 2001 Differential distribution of simple sequence repeats in eukaryotic genome sequences. Mol. Biol. Evol. **18:** 1161–1167.

Kay, M. A., J. Y. Zhang and M. Jacobs-Lorena, 1988 Identification and germline transformation of the ribosomal protein rp21 gene of Drosophila: complementation analysis with the Minute QIII locus reveals nonidentity. Mol. Gen. Genet. **213:** 354–358.

Kelley, R. L., and M. I. Kuroda, 1995 Equality for X chromosomes. Science **270:** 1607–1610.

Kimmerly, W., K. Stultz, S. Lewis, K. Lewis, V. Lustre *et al.*, 1996 A P1-based physical map of the Drosophila euchromatic genome. Genome Res. **6:** 414–430.

Kokoza, E. B., E. S. Belyaeva and I. F. Zhimulev, 1992 Localization of genes *ecs, dor* and *swi* in eight Drosophila species. Genetica **87:** 79–85.

Kraemer, C., B. Weil, M. Christmann and E. R. Schmidt, 1998 The new *DmX* from *Drosophila melanogaster* encodes a novel WD-repeat protein. Gene **216:** 267–276.

Krimbas, C. B., and J. R. Powell, 1992 *Drosophila Inversion Polymorphism.* CRC Press, Boca Raton, FL.

Laayouni, H., M. Santos and A. Fontdevila, 2000 Toward a physical map of *Drosophila buzzatii*: use of randomly amplified polymorphic DNA polymorphisms and sequence-tagged site landmarks. Genetics **156:** 1797–1816.

Lander, E. S., L. M. Linton, B. Birren, C. Nusbaum, M. C. Zody *et al.*, 2001 Initial sequencing and analysis of the human genome. Nature **409:** 860–921.

Lefevre, G., Jr., 1976 A photographic representation and interpretation of the polytene chromosomes of *D. melanogaster* salivary glands, pp. 31–66 in *The Genetics and Biology of Drosophila*, Vol. 1a, edited by M. Ashburner and E. Novitski. Academic Press, London.

Lemeunier, F., and F. Aulard, 1992 Inversion polymorphism in *Drosophila melanogaster*, pp. 339–405 in *Drosophila Inversion Polymorphism*, edited by C. B. Krimbas and J. R. Powell. CRC Press, Boca Raton, FL.

Lim, J. K., and M. J. Simmons, 1994 Gross chromosome rearrangements mediated by transposable elements in *Drosophila melanogaster*. Bioessays **16:** 269–275.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.

Llorente, B., A. Malpertuy, C. Neuvéglise, J. Montigny, M. Aigle *et al.*, 2000 Genomic exploration of the hemiascomycetous yeasts: comparative analysis of chromosome maps and synteny with *Saccharomyces cerevisiae*. FEBS Lett. **487:** 101–112.

Lowenhaupt, K., A. Rich and M. L. Pardue, 1989 Nonrandom distribution of long mono- and dinucleotide repeats in Drosophila chromosomes: correlations with dosage compensation, heterochromatin, and recombination. Mol. Cell. Biol. **9:** 1173–1182.

Lucchesi, J. C., and D. T. Suzuki, 1968 The interchromosomal control of recombination. Annu. Rev. Genet. **2:** 53–86.

Lunde, K., B. Biehs, U. Nauber and E. Bier, 1998 The *knirps* and *knirps-related* genes organize development of the second wing vein in Drosophila. Development **125:** 4145–4154.

Madueño, E., G. Papagiannakis, G. Remmington, R. D. C. Saunders, C. Savakis *et al.*, 1995 A physical map of the X chromosome of *D. melanogaster*: cosmid contigs and sequence tagged sites. Genetics **139:** 1631–1647.

Maier, D., B. M. Marte, W. Schafer, Y. Yu and A. Preiss, 1993 *Drosophila* evolution challenges postulated redundancy in the *E(spl)* gene complex. Proc. Natl. Acad. Sci. USA **90:** 5464–5468.

Mathiopoulus, K. D., A. della Torre, F. Santolamazza, V. Predazzi, V. Pretarca *et al.*, 1999 Are chromosomal inversions induced by transposable elements?: a paradigm from the malaria mosquito *Anopheles gambiae*. Parasitologia **41:** 119–123.

McClelland. A., D. F. Smith and D. M. Glover, 1981 Short intervening sequences close to the 5′ ends of the three Drosophila larval serum protein 1 genes. J. Mol. Biol. **153:** 257–272.

McGinnis, W., A. W. Shermoen and S. K. Beckendorf, 1983 A transposable element inserted just 5′ to a Drosophila glue protein gene alters gene expression and chromatin structure. Cell **34:** 75–84.

Mitas, M., 1997 Trinucleotide repeats associated with human disease. Nucleic Acids Res. **25:** 2245–2253.

Modolell, J., and S. Campuzano, 1998 The *achaete-scute* complex as an integrating device. Int. J. Dev. Biol. **42:** 275–282.

Mongelard, F., and V. G. Corces, 2001 Two insulators are not better than one. Nat. Struct. Biol. **8:** 192–194.

Montell, C., K. Jones, C. Zuker and G. Rubin, 1987 A second opsin gene expressed in the ultraviolet-sensitive R7 photoreceptor cells of *Drosophila melanogaster*. J. Neurosci. **7:** 1558–1566.

Montgomery, E. A., S. M. Huang, C. H. Langley and B. H. Judd, 1991 Chromosome rearrangement by ectopic recombination in *Drosophila melanogaster*: genome structure and evolution. Genetics **129:** 1085–1098.

Moore, H., P. W. Greenwell, C.-P. Liu, N. Arnheim and T. Petes, 1999 Triplet repeats form secondary structures that escape DNA repair in yeast. Proc. Natl. Acad. Sci. USA **96:** 1504–1509.

Muller, J. H., 1940 Bearings of the Drosophila work on systematics, pp. 185–268 in *New Systematics*, edited by J. Huxley. Clarendon Press, Oxford.

Müller, S., R. Stanyon, P. Finelli, N. Archidiacono and J. Wienberg, 2000 Molecular cytogenetic dissection of human chromosomes 3 and 21 evolution. Proc. Natl. Acad. Sci. USA **97:** 206–211.

Murphy, W. J., R. Stanyon and S. J. O'Brien, 2001 Evolution of mammalian genome organization inferred from comparative gene mapping. Genome Biol. **2:** 0005.1–0005.8.

Nadeau, J., and D. Sankoff, 1998a Counting on comparative maps. Trends Genet. **14:** 495–501.

Nadeau, J., and D. Sankoff, 1998b The lengths of undiscovered conserved segments in comparative maps. Mamm. Genome **9:** 491–495.

Nadeau, J. H., and B. A. Taylor, 1984 Lengths of chromosomal segments conserved since divergence of man and mouse. Proc. Natl. Acad. Sci. USA **81:** 814–818.

Natzle, J. E., A. S. Hammonds and J. W. Fristrom, 1986 Isolation of genes active during hormone-induced morphogenesis in Drosophila imaginal discs. J. Biol. Chem. **261:** 5575–5583.

Navarro, A., E. Betrán, A. Barbadilla and A. Ruiz, 1997 Recombination and gene flux caused by gene conversion and crossing over in inversion heterokaryotypes. Genetics **146:** 695–709.

Naveira, H., C. Pla and A. Fontdevila, 1986 The evolutionary history of *Drosophila buzzatii*. XI. A new method for cytogenetic hybrids of *Drosophila*. Genetica **71:** 199–212.

Neufeld, T. P., R. W. Carthew and G. M. Rubin, 1991 Evolution of gene position: chromosomal arrangement and sequence comparison of the *Drosophila melanogaster* and *Drosophila virilis sina* and *Rh4* genes. Proc. Natl. Acad. Sci. USA **88:** 10203–10207.

O'Brien, S. J., M. Menotti-Raymond, W. J. Murphy, W. G. Nash, J. Wienberg *et al.*, 1999 The promise of comparative genomics in mammals. Science **286:** 458–481.

Ohno, S., 1967 *Sex Chromosomes and Sex-Linked Genes*. Springer-Verlag, New York.

Orr, H. A., 1997 Haldane's rule. Annu. Rev. Ecol. Syst. **28:** 195–218.

Pardue, M. L., K. Lowenhaupt, A. Rich and A. Nordheim, 1987 (dC-dA)n (dG-dT)n sequences have evolutionary conserved chromosomal locations in Drosophila with implication for roles in chromosome structure and function. EMBO J. **6:** 1781–1789.

Paterson, J., and K. O'Hare, 1991 Structure and transcription of the *singed* locus of *Drosophila melanogaster*. Genetics **129:** 1073–1084.

Perkins, L. A., I. Larsen and N. Perrimon, 1992 *Corkscrew* encodes a putative protein tyrosine phosphatase that functions to transduce the terminal signal from the receptor tyrosine kinase torso. Cell **70:** 225–236.

Pletcher, M. T., B. A. Roe, F. Chen, T. Do, A. Do *et al.*, 2000 Chromosome evolution: the junction of mammalian chromosomes in the formation of mouse chromosome 10. Genome Res. **10:** 1463–1467.

Postlethwait, J. H., I. G. Woods, P. Ngo-Hazelett, Y. L. Yan, P. D. Kelly *et al.*, 2000 Zebrafish comparative genomics and the origins of vertebrate chromosomes. Genome Res. **10:** 1890–1902.

Powell, J. R., 1997 *Progress and Prospects in Evolutionary Biology: The Drosophila Model*. Oxford University Press, New York.

Puttagunta, R., L. A. Gordon, G. E. Meyer, D. Kapfhamer, J. E. Lamerdin *et al.*, 2000 Comparative maps of human 19p13.3 and mouse chromosome 10 allow identification of sequences at evolutionary breakpoints. Genome Res. **10:** 1369–1380.

Randazzo, F. M., M. A. Seeger, C. A. Huss, M. A. Sweeney, J. K. Cecil *et al.*, 1993 Structural changes in the *Antennapedia* complex of *Drosophila pseudoobscura*. Genetics **133:** 319–330.

Ranz, J. M., C. Segarra and A. Ruiz, 1997 Chromosomal homology and molecular organization of Muller's element D and E in the *Drosophila repleta* species group. Genetics **145:** 281–295.

Ranz, J. M., M. Cáceres and A. Ruiz, 1999 Comparative mapping of cosmids and gene clones from a 1.6 Mb chromosomal region of *Drosophila melanogaster* in three species of the distantly related subgenus Drosophila. Chromosoma **108:** 32–43.

Ranz, J. M., F. Casals and A. Ruiz, 2001 How malleable is the eukaryotic genome? Extreme rate of chromosomal rearrangements in the genus Drosophila. Genome Res. **11:** 230–239.

Rice, W. R., 1984 Sex chromosomes and the evolution of sexual dimorphism. Evolution **38:** 735–742.

Rizzon, C., G. Marais, M. Gouy and C. Biémont, 2002 Recombination rate and the distribution of transposable elements in the *Drosophila melanogaster* genome. Genome Res. **12:** 400–407.

Rubin, G. M., L. Hong, P. Brokstein, M. Evans-Holm, E. Frise *et al.*, 2000 A Drosophila complementary resource. Science **287:** 2222–2224.

Ruiz, A., and M. Wasserman, 1993 Evolutionary cytogenetics of the *Drosophila buzzatii* species complex. Heredity **70:** 582–596.

Russo, C. A. M., N. Takezaki and M. Nei, 1995 Molecular phylogeny and divergence times of Drosophilid species. Mol. Biol. Evol. **12:** 391–404.

Sambrook, J., E. F. Fritsch and T. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schneuwly, S., A. Kuroiwa and W. J. Gehring, 1987 Molecular analysis of the dominant homeotic *Antennapedia* phenotype. EMBO J. **6:** 201–206.

Schoen, D. J., 2000 Comparative genomics, marker density and statistical analysis of chromosome rearrangements. Genetics **154:** 943–952.

Schulze, D. H., and C. S. Lee, 1986 DNA sequence comparison among closely related Drosophila species in the mulleri complex. Genetics **113:** 287–303.

Segarra, C., E. R. Lozovskaya, G. Ribó, M. Aguadé and D. L. Hartl, 1995 P1 clones from *Drosophila melanogaster* as markers to study the chromosomal evolution of Muller's A element in two species of the *obscura* group of Drosophila. Chromosoma **104:** 129–136.

Segarra, C., G. Ribó and M. Aguadé, 1996 Differentiation of Muller's elements D and E in the *obscura* group of Drosophila. Genetics **144:** 139–146.

Seoighe, C., N. Federspiel, T. Jones, N. Hansen, V. Bivolarovic *et al.*, 2000 Prevalence of small inversions in yeast gene order evolution. Proc. Natl. Acad. Sci. USA **97:** 14433–14437.

Smith, D. F., A. McClelland, B. N. White, C. F. Addison and D. M. Glover, 1981 The molecular cloning of a dispersed set of developmentally regulated genes which encode the major larval serum protein of *D. melanogaster.* Cell **23:** 441–449.

Spicer, G. S., 1988 Molecular evolution among some Drosophila species groups as indicated by two-dimensional electrophoresis. J. Mol. Evol. **27:** 250–260.

Stuckenholz, C., Y. Kageyama and M. I. Kuroda, 1999 Guilt by association: non-coding RNAs, chromosome-specific proteins and dosage compensation in Drosophila. Trends Genet. **15:** 454–458.

Sturtevant, A. H., and E. Novitski, 1941 The homologies of the chromosome elements in the genus Drosophila. Genetics **26:** 517–541.

Sun, H.-F. S., C. W. Ernst, M. Yerle, P. Pinton, M. F. Rothschild *et al.*, 1999 Human chromosome 3 and pig chromosome 13 show complete synteny conservation but extensive gene-order differences. Cytogenet. Cell Genet. **85:** 273–278.

True, J. R., J. M. Mercer and C. C. Laurie, 1996 Differences in crossover frequency and distribution among three sibling species of Drosophila. Genetics **142:** 507–523.

Tudor, M., A. Mitchelson and K. O'Hare, 1996 A 1.5 kb repeat sequence flanks the *suppressor of forked* gene at the euchromatin-heterochromatin boundary of the *Drosophila melanogaster* X chromosome. Genet. Res. **68:** 191–202.

Vieira, J., C. P. Vieira, D. L. Hartl and E. R. Lozovskaya, 1997a A framework physical map of *D. virilis* based on P1 clones: applications in genome evolution. Chromosoma **106:** 99–107.

Vieira, J., C. P. Vieira, D. L. Hartl and E. R. Lozovskaya, 1997b Discordant rates of chromosome evolution in the *Drosophila virilis* species group. Genetics **147:** 223–230.

Wang, P. J., J. R. McCarrey, F. Yang and D. C. Page, 2001 An abundance of X-linked genes expressed in spermatogonia. Nat. Genet. **27:** 422–426.

Waring, G. L., and J. C. Pollack, 1987 Cloning and characterization of a dispersed, multicopy, X chromosome sequence in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **84:** 2843–2847.

Wasserman, M., 1992 Cytological evolution of the *Drosophila repleta* species group, pp. 455–552 in *Drosophila Inversion Polymorphism*, edited by C. B. Krimbas and J. R. Powell. CRC Press, Boca Raton, FL.

Wharton, L. T., 1942 Analysis of the *repleta* group of Drosophila. University of Texas Pub. **4228:** 23–52.

Wright, T. R. F., 1996 Phenotypic analysis of the *dopa decarboxylase* gene cluster mutants in *Drosophila melanogaster.* J. Hered. **87:** 175–190.

Yi, S., and B. Charlesworth, 2000 A selective sweep associated with a recent gene transposition in *Drosophila miranda.* Genetics **156:** 1753–1763.

Communicating editor: D. Charlesworth

APPENDIX

Cytological localization of the 145 markers hybridized in this study on the polytene chromosomes of *D. repleta* (along with 17 markers mapped by other authors)

	Hybridization signals	
Marker	*D. melanogaster*	*D. repleta*
23E12	X(1A)	X(A3i-k)
125H10	X(1B1-4)	X(C3b, B3ab)
198A6 (sc)	X(1B1-4)	X(B3ab)
sc[a]	X(1B3)	X(B3ab)[b]
165H7	X(1B3-7)	X(B3ab)
171D11	X(1B2-7)	X(A4f, B3ab, F4b)
65F1	X(1B7-10)	X(F4b)
br[c]	X(2B3-5)	X(A1)
dor[c]	X(2B6)	X(A1)
hfw[c]	X(2B6)	X(A1)
129E12 (arm)	X(2B9-16)	X(A2d, B3f, C4a)[b]
arm	X(2B15)	X(C4a)
csw	X(2D3)	X(D3d)
28C2	X(2E)	X(A2d, B1h, D2f-g)

(*continued*)

APPENDIX

(Continued)

Marker	Hybridization signals	
	D. melanogaster	*D. repleta*
156H1[d]	X(3A)	X(F1g)
155E2	X(3B2-4)	X(B1e)
w	X(3C2)	X(B4a)
crb (secondary signal)[e]	X(3B)	X(B3f)
CG6450	X(4A2)	X(C2d-e)
peb	X(4C5-6)	X(B1a-b)
65G11	X(4D1-7)	X(A4b, C3a, E1a, F1c-f)[b]
CG4165	X(4F9)	X(C3b)
142A8	X(5B1-10)	X(G1f-g)
Act5C[e]	X(5C2-5)	X(D2h-3a)
143G11 (CG3585, Ubi-p5E)	X(5D1-E8)	X(A4f, C2h)
CG3585	X(5D6-8)	X(A4f)
Ubi-p5E[e]	X(5E)	X(C3a)
Fum[a]	X(5D5)	X(D3b-e)[b]
173C3 (Fum)	X(6B1-C13)	X(D3f-g)
60H5	X(6F1-7A8)	X(C1g-h)
Sxl[a]	X(6F5)	X(C1g-h)[b]
125A5 (Sxl)	X(6F1-7A8)	X(C1g-h)[b]
DS00188	X(7B1-6)	X(E1d)
190F5	X(7D1-22)	X(A4a)[b]
sn	X(7D)	X(C1c-d)
DS09021	X(8B5-8)	X(E3b)
rdgA	X(8C4-7)	X(E3d)
164F6	X(8D1-12)	X(E3g)
190E8	X(8D1-12)	X(D2c-d)
Yp2	X(9A4-5)	X(F1b)
Yp1	X(9A4-5)	X(F1b)
186C8	X(9F1-13)	X(D1b)
36A5	X(9F1-10A11)	X(D1b)
Lsp1alpha	X(10A)	2(D3e)
11G9	X(10A1-2)	X(B1e)
DS02496	X(10A1-2)	X(A1e-f)
RpII215	X(10C4-5)	X(A1d-e)
CG1559	X(10F6-7)	X(G2b)
167B1	X(11B1-19)	X(A3b)
CG11056	X(11B14)	X(A4d)
61A7	X(11B1-C4)	X(A3b)
144B11	X(12A1-10)	X(D3e)[b]
CG1716	X(12A)	X(F3g), 4(C3cd)
171A8	X(12E1-11)	X(F2g, F2j)
191A7	X(12D1-4)	X(C3f)
176C7	X(13E1-18)	X(C3a)
sd	X(13F1-3)	X(C4c-e)
8C2 (sd)	X(13D1-5)	X(D1c-d)
192D9	X(14F1-6)	X(G2i)[b]
116C6	X(14E1-4)	X(F4a)
143D3	X(14F1-15A11)	X(B2e-f)
148H11	X(15A1-11)	X(H1f)
DS05647	X(15E1-4)	X(D1g)
194F2	X(15F1-9)	X(E3e)
DS04269	X(15F6-9)	X(F2h)
96C3	X(16C1-10)	X(B1b)
56F9	X(16C1-10)	X(B1b, B2b)
199E12	X(16E1-F8)	X(A3k)[b]
114E10	X(16F1-8)	X(F2b)
37C10	X(17C1-7)	X(E3a)

(*continued*)

APPENDIX

(Continued)

Marker	Hybridization signals	
	D. melanogaster	*D. repleta*
DS07456	X(17D1-6)	X(A4f)
CG7282	X(17E4)	X(B1g)
CG7358	X(17E6)	X(E4c-d)
108C8	X(18C1-9)	X(A2a)[b]
60H9	X(18C1-9)	X(A2a)
94E9	X(18D1-13)	X(D3j)
173H11	X(18D1-13)	X(B2f-g)
Zw	X(18D)	X(B1h)
163H9	X(19E1-8)	X(B1b-c)
DS03594	X(19D1-3)	X(B1c-d, F3c)
153F10	X(20A1-C3)	X(G2d)
DS02945	X(20A1-2)	X(C1e)
174F6	X(20A1-C3)	4(G5d)
13F10[d]	3L(61B1-3)	4(B1i)
kls[f]	3L(61C4)	4(A1g)[b]
DS07291	3L(61F)	4(A2c)
DS02777	3L(62A10)	4(E1f)
DS04710	3L(62B11)	4(E1f, A1a)
DS05034	3L(63A1-2)	4(G3f-g)
Hsp83	3L(63C1)	4(E2b)
DS01859	3L(63C5)	4(B3f, C1h)
DS07627	3L(63D1-3)	4(C1h)
DS03985	3L(63E1)	4(B2e)
Ubi-p63E[e]	3L(63F2-4)	4(B1d)
ImpL2	3L(64B2)	4(A3a)
DS00926	3L(64D1-2)	4(D3e)
DS02635	3L(64F1-2)	4(E1c)
DS08881	3L(65A6)	4(D4e)
DS05402	3L(65B5)	4(D4d-e)
DS08305	3L(65C1)	4(D4d)
DS00374	3L(65D)	4(C2a)
DS06194	3L(65E1)	4(C1i-C2a)
DS05040	3L(66A1-2)	4(B3e-f)
CG7185	3L(66C)	4(C2a)
ImpE1	3L(66C1-3)	4(B3a)
DS00606	3L(66C8-D2)	4(B3a)
DS00529	3L(66D1-6)	4(C1a)
DS04988	3L(66D10-11)	4(C1b)
DS07824	3L(67A1-2)	4(A5a)
Hsp22-Hsp26	3L(67B1)	4(D1a)
Hsp23-Hsp27	3L(67B1)	4(D1a)
DS03929 (Hsp 22-26, Hsp 23-27)	3L(67B2-7)	4(D5d)
DS03212	3L(67B7-8)	4(E2c)
DS00256	3L(67C1-2)	4(D5c)
DS04653	3L(68A1-2)	4(G3a)
Sod	3L(68A8-9)	4(F4c)
DS01438	3L(68C12-13)	4(F4c-d)
DS05904	3L(68F1-2)	4(F4a)
DS08585[d]	3L(69A)	4(C3a)
CG10632[f]	3L(69C4-8)	4(C3g)[b]
DS08512 (caup, ara)	3L(69D1)	4(C3g)
caup	3L(69D3)	4(C3g)
ara	3L(69D3)	4(C3g)
DS06094	3L(69E6-7)	4(F3b)
DS02826	3L(69F3-7)	4(F1b, F3c)
DS03501	3L(70B1-3)	4(G1e, G4a)

(*continued*)

APPENDIX

(Continued)

Marker	Hybridization signals	
	D. melanogaster	*D. repleta*
Hsc70-1[e]	3L(70C)	4(F4a)
DS00192	3L(70E4-5)	4(G2b-c)
DS04421	3L(70F1-6)	4(G1g)
DS07775	3L(71A1-2)	4(G4c)
DS01389	3L(71D1-2)	4(D1a-c)
DS02456	3L(71F1-2)	4(F2f)
DS03598	3L(72C1-D6)	4(C3a,C3i)
Pgm[a]	3L(72D1-5)	4(A1a-g)[b]
DS01052	3L(72F1-2)	4(A4de)
DS05180	3L(73A2-3)	4(A4b)
tra[e]	3L(73A8-9)	4(A3c)
DS04370 (tra)	3L(73B5-C1)	4(E2d-e)
Rh4	3L(73D3-5)	4(F2g-h)
sina	3L(73D)	4(B4c)
DS00383	3L(73D4-E2)	4(B4c)
DS00052	3L(74A5-B1)	4(B4e)
Eip74EF	3L(74D2-5)	4(C1e-f)
DS03359 (Eip74EF)	3L(74E2-F1)	4(C1e-f)
DS07350	3L(75A1-2)	4(C1d)
DS05726	3L(75C1-2)	4(F1f)[b]
DS01293	3L(75E2-7)	4(E4g)
DS07819	3L(76A2-4)	4(E4c)
DS00004[d]	3L(76B3-4)	4(E5e)
DS01645	3L(76F1-2)	4(F4i)
DS00288	3L(77A4-B1)	4(G1b)
DS01393	3L(77B4-C1)	4(E4d-e)
DS01369	3L(77E)	4(E3a)
kni[e]	3L(77E1-2)	4(E3a)
DS00239	3L(77E4-F1)	4(E3a)
DS05090	3L(78A1-2)	4(A3d)
DS00088	3L(78B1-2)	4(G2d, G3d)
DS09130	3L(78E1-F2)	4(E4f)
DS02213	3L(79A3-4)	4(F4g)
Act79B[e]	3L(79B)	4(F1c)
DS03596	3L(79D4)	4(G4a)
DS03402	3L(80A1-2)	4(A1f)

Genes known to be included in cosmids or P1 phages are enclosed in parentheses after the clone name.
[a] Naveira *et al.* (1986); H. Naveira, personal communication.
[b] Clones hybridized in *D. buzzatii.*
[c] Kokoza *et al.* (1992).
[d] Clone must be mislabeled as shown by the control hybridization.
[e] Ranz *et al.* (1997, 1999).
[f] Laayouni *et al.* (2000).