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Toward a Physical Map of *Drosophila buzzatii*: Use of Randomly Amplified Polymorphic DNA Polymorphisms and Sequence-Tagged Site Landmarks

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Manuscript received December 7, 1999
Accepted for publication August 21, 2000

ABSTRACT

We present a physical map based on RAPD polymorphic fragments and sequence-tagged sites (STSs) for the *repleta* group species *Drosophila buzzatii*. One hundred forty-four RAPD markers have been used as probes for *in situ* hybridization to the polytene chromosomes, and positive results allowing the precise localization of 108 RAPDs were obtained. Of these, 73 behave as effectively unique markers for physical map construction, and in 9 additional cases the probes gave two hybridization signals, each on a different chromosome. Most markers (68%) are located on chromosomes *2* and *4*, which partially agree with previous estimates on the distribution of genetic variation over chromosomes. One RAPD maps close to the proximal breakpoint of inversion $2z^3$ but is not included within the inverted fragment. However, it was possible to conclude from this RAPD that the distal breakpoint of $2z^3$ had previously been wrongly assigned. A total of 39 cytologically mapped RAPDs were converted to STSs and yielded an aggregate sequence of 28,431 bp. Thirty-six RAPDs (25%) did not produce any detectable hybridization signal, and we obtained the DNA sequence from three of them. Further prospects toward obtaining a more developed genetic map than the one currently available for *D. buzzatii* are discussed.

A common tenet in evolutionary biology is that an ultimate understanding of evolution by natural selection requires an integrated approach from genetics and ecology. Unfortunately, there seems to be an increasing gap between our current knowledge from very well-studied genomes and the ecological scenarios where these genomes have evolved. As a noteworthy example, compare the massive amount of information in recent releases of the FlyBase (FlyBase Consortium 1999)—the comprehensive database for the fruitfly—with the number of entries for Drosophila in Endler's (1986, pp. 129–153) broad review of direct demonstrations of selection on naturally occurring genetic variation: just one for *Drosophila buzzatii* and two for *D. melanogaster*! Because of this empirical restriction, we need a reasonable model where both approaches to understanding evolution can be successfully combined.

Perhaps the best-characterized ecology of any Drosophila group is for the *repleta* group species, and we agree with Powell (1997, p. 149) in that "anyone looking for a system to connect ecology with genetics would do well to consider the *repleta* group." Particularly, *D. buzzatii* provides a valuable model system for studies in natural populations and evolutionary genetics. Thus, this species is restricted to the cactus niche, feeding and breeding in rotting tissues, but has a worldwide distribution (Carson and Wasserman 1965; Barker 1977; Fontdevila *et al.* 1981, 1982; Haouas *et al.* 1984). A substantial number of articles in ecological genetics (*e.g.*, Barker and East 1980; Barker 1982; Santos *et al.* 1989; Thomas and Barker 1990; Quezada-Díaz *et al.* 1992; Santos 1994), life-history evolution (Ruiz *et al.* 1986; Hasson *et al.* 1991; Santos *et al.* 1992; Barbadilla *et al.* 1994; Betrán *et al.* 1998), quantitative genetics (Prout and Barker 1989; Ruiz *et al.* 1991; Thomas and Barker 1993; Leibowitz *et al.* 1995; Santos 1996), thermal adaptation (Krebs and Loeschcke 1996, 1997, 1999; Imasheva *et al.* 1997), colonization (Fontdevila *et al.* 1981, 1982; Halliburton and Barker 1993; Rossi *et al.* 1996), and speciation (Naveira and Fontdevila 1986; 1991a,b) have focused on *D. buzzatii*. Conversely to *D. melanogaster*, this wealthy state of affairs markedly contrasts with a paucity of molecular markers in *D. buzzatii*, still restricted to a few allozymes (Schafer *et al.* 1993; Betrán *et al.* 1995). [A molecular marker is defined here as "any genetic variant that allows scoring of conspecific individuals at the molecular level." This is a somewhat narrower definition than that provided by King and Stansfield (1997) for a genetic marker, but is operationally and implicitly used in evolutionary biology (Avise 1994) and quantitative genetics (Lynch and Walsh 1998).]

To overcome this deficiency, here we present the first extensive effort to map by *in situ* hybridization to the polytene chromosomes of *D. buzzatii* a large number (144) of reproducible randomly amplified polymorphic DNA (RAPD; Welsh and McClelland 1990; Williams

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et al. 1990) markers. RAPDs have been successfully applied to the construction of linkage maps in a variety of organisms (*e.g.*, Reiter *et al.* 1992; Postlethwait *et al.* 1994; Hunt and Page 1995; Dimopoulos *et al.* 1996) and are becoming a frequently used tool in population and evolutionary genetics (Smith *et al.* 1994; de Zande and Bijlsma 1995; Apostol *et al.* 1996; Espinasa and Borowsky 1998).

In addition to convenience for recombination mapping, RAPDs can provide sequence-tagged sites (STSs; Olson *et al.* 1989) that serve as physical entry points to the genome. STSs can also be a rich source for detecting previously undescribed potential genes even in very well-studied genomes (Louis *et al.* 1997). We therefore have determined 39 STS landmarks from cloned RAPD sequences, and all sequences were checked against both nucleic acid and protein databases for potential matches. These STSs also allow us to roughly estimate the overall base composition of the *D. buzzatii* genome. The physical map obtained comprises a total of 73 effectively unique RAPD markers (39 of these are STSs), together with 9 RAPDs that gave two hybridization signals, each on a different chromosome. The results obtained from the combined use of different techniques allow the first comprehensive approach to the genome of *D. buzzatii*. We hope the information provided here will be an important tool for further development of a reasonably saturated genetic map in this species.

MATERIALS AND METHODS

Fly material: *D. buzzatii* flies were collected from a natural population in an abandoned *Opuntia ficus-indica* plantation at Carboneras on the Mediterranean coast of Spain (Almería; 37° N, 1° 9′ W; see Ruiz *et al.* 1986 for details). Between the 10th and 12th of September 1993, 36 rotting Opuntia cladodes were collected, placed individually in transparent plastic containers on a bed of sand, closed with a fine-meshed fabric, and kept at room temperature (22–27°) in the makeshift laboratory near the field site. From the adult flies that emerged from 28 rots, a high number of isofemale strains were established by pairwise mating in vials (2 × 8 cm, with 5 ml of food) of virgin females and males. The isofemale strains were maintained at 23° by one single brother-sister mating for the first ~18 generations and full-sib matings (4–8 mating pairs per vial) thereafter and passed through a minimum of ~36 generations before RAPD screening. Therefore, the probability that a neutral allele was still segregating in any particular isofemale strain is practically negligible (see, *e.g.*, Gale 1990). The population at Carboneras is polymorphic for the two common cosmopolitan *2st* and *2j* and for the two rare cosmopolitan $2jz^3$ and $2jq^7$, second-chromosome arrangements, as well as for the rare cosmopolitan *4st* and *4s* (Fontdevila *et al.* 1981; for a description see Ruiz *et al.* 1984).

DNA isolation: DNA was isolated from individual males from each isofemale strain. The following protocol is a modification of that described in Latorre *et al.* (1986). Each fly was homogenized in a 1.5-ml microcentrifuge tube containing 160 μl of 10 mm Tris/60 mm NaCl/5% (wt/vol) sucrose/10 mm EDTA, pH 7.8. One hundred microliters of 1.25% SDS/300 mm Tris/5% sucrose/10 mm EDTA, pH 9, were then added. The mixture was incubated at 65° for 30 min, after which 60 μl of 5 m potassium acetate was added and the mixture was kept at −20° for 20 min. After centrifugation for 15 min in an Eppendorf centrifuge, the supernatant was added to 1 volume of 2-propanol and left standing at room temperature for 5 min, which was followed by a 10-min Eppendorf centrifugation. The pellet was washed with 70% ethanol. Residual ethanol was removed by drying the precipitate in a desiccator for 5 min, after which the DNA was resuspended in 100 μl of sterile distilled water.

DNA amplifications: A set of 78 random decamer oligonucleotides purchased from Genosys Biotechnologies Inc. (Cambridge, UK) and 5 from Operon Technologies Inc. (Alameda, CA) were used as single primers for the amplification of RAPD sequences. Primers are listed in Table 1 as designated by the suppliers.

The conditions reported by Williams *et al.* (1990) for creating RAPD markers by PCR were optimized for use with *D. buzzatii* template DNA. All reaction volumes were 25 μl, overlayered with 50 μl of light mineral oil (Sigma Chemical Co., St. Louis). Each reaction consisted of 1× activity buffer (GIBCO BRL, Gaithersburg, MD), 1.6 mm $MgCl_2$, 200 μm of each dNTP (Boehringer Mannheim, Indianapolis), 400 nm primer, template DNA (~30–40 ng), and 0.8 units of Taq polymerase (GIBCO BRL). Only one primer and one genomic DNA sample were added to any single reaction. Amplification was achieved in an MJ Research Inc. (Watertown, MA) thermocycler programmed as follows: a preliminary 5-min denaturation at 94°; 45 cycles of 30 sec at 94° (denaturation), 1 min at 35° (anneal), and 1 min at 72° (extension); and a final extension at 72° for 5 min followed by storage at 4°. Electrophoresis was performed in 1.4% agarose gels (SeaKem) with Tris-HCl acetate/EDTA (TAE) buffer for 5 hr at 70 V, constant voltage. Reaction products were analyzed alongside small molecular weight marker VI (Boehringer Mannheim). Ethidium bromide-stained gels (0.5 μg/ml) were visualized on a UV transilluminator and photographed with a Polaroid camera or digitalized with a Bio-Print image management system. After testing for polymorphism and reproducibility (see below), the RAPD bands chosen as probes were gel purified, reamplified using the same decameric primer that identified the RAPD polymorphism, and labeled for *in situ* hybridization.

Polytene chromosome preparation and *in situ* hybridization: Probes (300 ng–1 μg DNA) were labeled with digoxygenin-11-dUTP by the random primer method using the Boehringer Mannheim labeling kit, and the total yield from the labeling reaction (500 ng–2 μg) was quantified according to the instructions supplied by the manufacturer. Third instar larvae were grown at low densities at 18° in a modified version of David's killed-yeast culture medium (David 1962). Salivary gland chromosomes suitable for *in situ* hybridization were prepared according to Labrador *et al.* (1990). Prehybridization, hybridization, and detection were carried out as described by de Frutos *et al.* (1989). Hybridization temperature was 37°. Chromosomes were observed by phase contrast with a Zeiss Axioscope photomicroscope at ×400 magnification and digitalized with a Bio-Print image management system.

Chromosome mapping: The karyotype of most repleta species, including *D. buzzatii*, consists of five telocentric chromosomes (*1 = X, 2, 3, 4, 5*) and a dot (*6*) chromosome (Wasserman 1992). Hybridization signals were localized on the polytene chromosomes using the *D. repleta* (Wharton 1942) and *D. buzzatii* (Ruiz *et al.* 1982; Ruiz and Wasserman 1993) cytological maps. The maps of *D. buzzatii* are cut-and-paste reconstructions of the *D. repleta* map according to the sequence of inversions proposed for their respective phylogenies. The molecular organizations of Mueller/Sturtevant/Novitski chromosomal elements *D* (= *4*) and *E* (= *2*) in *D. repleta* and *D. buzzatii* (see Powell 1997, p. 307—but note that exact correspondence of chromosomal arms in *D. hydei* is misplaced and readers should refer to Loukas and Kafatos 1986 for exact homologies) has been compared recently by *in situ* hy-

TABLE 1

Nucleotide sequences of random primers G-50.01 to G-80.20 and OPA-07 to OPA-17

Primer	Sequence	Primer	Sequence	Primer	Sequence	Primer	Sequence
G-50.01	5′GTGCAATGAG-3′	G-60.05	5′-GTCCTCAACG-3′	G-70.08	5′-CTGTACCCCC-3′	G-80.12	5′-CGACGCGTGC-3′
G-50.02	5′-CAATGCGTCT-3′	G-60.06	5′-CTACTACCGC-3′	G-70.09	5′-TGCAGCACCG-3′	G-80.13	5′-ACCCGTCCCC-3′
G-50.03	5′-AGGATACGTG-3′	G-60.07	5′-GAGTCACTCG-3′	G-70.10	5′-CAGACACGGC-3′	G-80.14	5′-GCAGCTCCGG-3′
G-50.04	5′-TCCCTTTAGC-3′	G-60.08	5′-GTCCTCAGTG-3′	G-70.11	5′-GTCTCGTCGG-3′	G-80.15	5′-CGAGACGGGC-3′
G-50.05	5′-CGGATAACTG-3′	G-60.09	5′-CGTCGTTACC-3′	G-70.12	5′-GGCCTACTCG-3′	G-80.16	5′-ACCGCCTCCC-3′
G-50.06	5′-AGGTTCTAGC-3′	G-60.10	5′-GCAGACTGAG-3′	G-70.13	5′-GTGTAGGGCG-3′	G-80.17	5′-GCAGGTCGCG-3′
G-50.07	5′-TCCGACGTAT-3′	G-60.21	5′-GAGTGTCTCG-3′	G-70.14	5′-CGGGTCGATC-3′	G-80.18	5′-GGCCACAGCG-3′
G-50.08	5′-GGAAGACAAC-3′	G-60.22	5′-CACATAGCGC-3′	G-70.15	5′-GCCCTCTTCG-3′	G-80.19	5′-ACGCCCTGGC-3′
G-50.09	5′-AGAAGCGATG-3′	G-60.23	5′-CGAAGCGATC-3′	G-70.16	5′-CAGGGGCATC-3′	G-80.20	5′-CGCGAACGGC-3′
G-50.10	5′-CCATTTACGC-3′	G-60.24	5′-CCCTCATCAC-3′	G-70.17	5′-GAGACCTCCG-3′	OPA-07	5′-GAAACGGGTG-3′
G-50.21	5′-ACGCTACATC-3′	G-60.25	5′-CCTGTTAGCC-3′	G-70.18	5′-GGCCTTCAGG-3′	OPA-10	5′-GTGATCGCAG-3′
G-50.22	5′-CGAAACAGTC-3′	G-60.26	5′-GCAGCTCATG-3′	G-70.19	5′-GCTCTCACCG-3′	OPA-11	5′-CAATCGCCGT-3′
G-50.23	5′-CTTACACTTG-3′	G-60.27	5′-CGCTTGCTAG-3′	G-70.20	5′-TGCACGGACG-3′	OPA-14	5′-TCTGTGCTGG-3′
G-50.24	5′-GTTAGTGGCA-3′	G-60.28	5′-GAACCTACGG-3′	G-80.01	5′-GCACCCGACG-3′	OPA-17	5′-GACCGCTTGT-3′
G-50.25	5′-ATCTGAGGAG-3′	G-60.29	5′CTAGCTGAGC-3′	G-80.03	5′-CCATGGCGCC-3′		
G-50.26	5′-AAGATAGCGG-3′	G-60.30	5′-GAGCAGGCTG-3′	G-80.04	5′-CGCCCGATCC-3′		
G-50.27	5′-CCTATCCGTT-3′	G-70.01	5′-CATCCCGAAC-3′	G-80.05	5′-ACCCCAGCCG-3′		
G-50.28	5′-GATTGCGTTC-3′	G-70.02	5′-CAGGGTCGAC-3′	G-80.06	5′-GCACGGAGGG-3′		
G-50.30	5′-TGCTGTGAAC-3′	G-70.03	5′-ACGGTGCCTG-3′	G-80.07	5′-GCACGCCGGA-3′		
G-60.01	5′-CGCAGTACTC-3′	G-70.04	5′-CGCATTCCGC-3′	G-80.08	5′-CGCCCTCAGC-3′		
G-60.02	5′-GTCCTACTCG-3′	G-70.05	5′-GAGATCCGCG-3′	G-80.09	5′-GCACGGTGGG-3′		
G-60.03	5′-CTACACAGGC-3′	G-70.06	5′-GGACTCCACG-3′	G-80.10	5′-CGCCCTGGTC-3′		
G-60.04	5′-GTCCTTAGCG-3′	G-70.07	5′-ATCTCCCGGG-3′	G-80.11	5′-GCAGCAGCCG-3′		

The 44 primers that rendered polymorphic and reproducible bands are indicated by underlining (see text for details). G-50.01 to G-80.20, Gynosys Biotechnologies Inc; OPA-07 to OPA-17, Operon Technologies Inc.

bridization (Ranz *et al.* 1997). Within the limits of potential resolution, Ranz *et al.* (1997) concluded that the formerly proposed cytogenetic relationships between both species seem to be consistent with the new results.

DNA sequencing: Thirty-nine single-signal RAPD markers (see below) were converted to STSs (Olson *et al.* 1989). Gel-purified RAPD fragments (10–100 ng) were directly cloned into pGEM-T Vector (Promega, Madison, WI). DNA mini-preparations were made from positive clones of transformed JM109 *Escherichia coli* cells. The DNA sequences were determined by the dideoxy method (Sanger *et al.* 1977) using an ALF sequencer (Pharmacia Biotech, Piscataway, NJ). Nucleotide sequences were determined on both DNA strands and included ~80 nucleotides of the T vector flanking the cloning site.

Nucleic acid searches were performed using the BLAST program (Altschul *et al.* 1997). BLASTN was used to search the nucleic acid database, BLASTX to search the protein database with the putative translations of the STSs in all six frames, and ORF Finder program to look for potential open reading frames (ORFs). Alignments were also obtained using the default option of the program CLUSTAL W (version 1.6) (Thompson *et al.* 1994).

RESULTS AND DISCUSSION

RAPD products and RAPD product profiles: Forty-four random decamer oligonucleotides (Table 1) yielded reproducible and polymorphic DNA fragments. A fragment was considered polymorphic when absent in at least 1 individual out of 14 from different (*i.e.*, independent) isofemale strains, *i.e.*, when the recessive (absence) allele was at an average frequency of at least 7% in the natural population (a more restrictive criterion than the standard 5 or 1% used in population genetics; see, *e.g.*, Hedrick 1985). Repeating the amplification using a set of five or more individuals that had rendered polymorphic bands tested the reproducibility of the different profiles. A particular band was considered as reproducible when the profiles from the two independent amplifications were consistent in all individuals tested.

Those 44 primers generated 547 scorable marker bands (an average of 12.4 bands per primer), of which 257 (47%) were polymorphic. RAPD reproducibility (see above) was obtained for 144 (56%) fragments, which were used as probes for *in situ* hybridization. RAPDs were named according to the decameric primer that identified the RAPD polymorphism, followed by a digit that increases as the relative mobility of the band increases. Figure 1 shows a typical example of RAPD products. A negative but nonsignificant correlation between the G + C content of the decameric primer (as given by the first number in the primers from Genosys Biotechnologies, Cambridge, UK) and the number of polymorphic bands scored was observed (Spearman $r_S = -0.302$; $P = 0.055$). On the other hand, there was a positive and statistically significant correlation between the G + C content of the primer and the fraction of polymorphic bands that were reproducible ($r_S = 0.453$; $P = 0.003$).

Positive hybridizations to the polytene chromosomes:

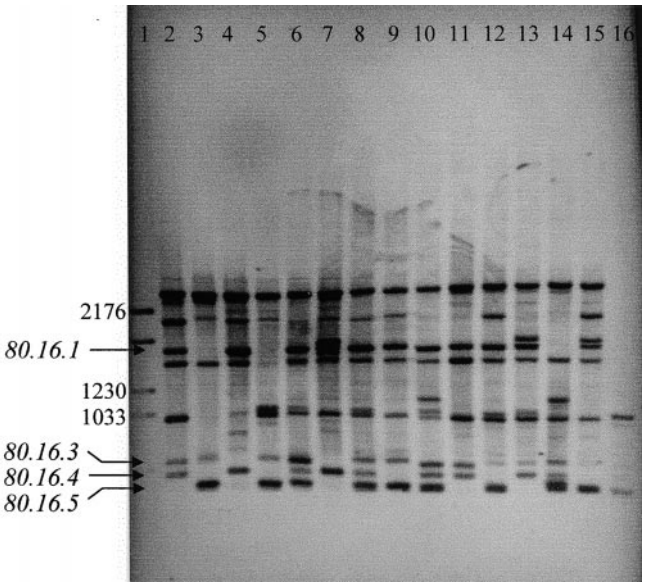

Figure 1.—RAPD profile for the decameric primer G-80.16. Lanes 2–15 are the PCR amplification products from individual template DNA samples coming from 14 independent isofemale strains of *D. buzzatii*. Lane 1 indicates the molecular weight standards, and their sizes are given on the left-hand side (in base pairs). Lane 16 is the negative control. Polymorphic and reproducible RAPD bands used as probes for *in situ* hybridization are indicated by arrows.

In situ hybridizations were routinely carried out using a *D. buzzatii* strain fixed for *2st* and *4st* gene arrangements. A total of 108 RAPDs produced a single or multiple signals (up to 15), and Table 2 gives the hybridization sites on the chromosomes from the salivary glands. Sixty-three RAPDs gave a single and consistently detectable hybridization signal that must correspond to the site of the polymorphic locus, and in 10 additional cases there were one or two extra secondary signals on the same or different chromosomes that were absent in some preparations. No variation in signal localization was ever detected among the several nuclei examined for a given probe. Hence, a total of 73 RAPDs with an average length of 942 bp (aggregate map length of ~69 kb) were considered to behave as effectively unique and to be valuable as markers for physical map construction. Fourteen RAPDs gave two primary signals, and in nine cases these signals were located on different chromosomes, thus potentially increasing the number of useful markers for further recombinational maps. Figure 2, a–e, shows a picture of *D. buzzatii* polytene chromosomes indicating the cytological positions of the 73 primary-singled signal RAPDs, together with the 9 primary-doubled signal RAPDs on different chromosomes (boldface type).

Two RAPDs map close to known inversion breakpoints, and they were converted to STSs for further analyses (see below). RAPD *70.18.1* maps on *2*(F1d) (Figure 2b), close to the proximal breakpoint of inversion $2z^3$ that has been previously assigned to *2*(F1c)

TABLE 2

Localization by *in situ* hybridization on the salivary gland chromosomes of *D. buzzatii* of the 108 RAPDs used as probes

Primer	RAPD	Size (bp)	Hybridization signal	
			Primary	Secondary
G-60.09	*60.09.4*	640	*X*(C3c)	
G-70.01	*70.01.4*	610	*X*(E3c) + *4*(F3a)	
G-70.10	*70.10.3*	735	*X*(centromere) + *4*(B2c)	
G-70.12	*70.12.2*	900	*X*(B4c) + *4*(C3d)	
G-70.13	*70.13.2*	1365	*X*(E4b)	
G-80.13	*80.13.3*	940	*X*(B1a)	*2*(D4c)
G-50.22	*50.22.1*	595	*2*(B2d-f) + *3*(F2e)	
G-50.30	*50.30.1*	551	*2*(G3a-b)	
G-60.03	*60.03.2*	1630	*2*(B2d-f)	
G-60.03	*60.03.5*	1000	*2*(B2d-f)	
G-60.03	*60.03.7*	556	*2*(E2a)	
G-60.03	*60.03.8*	450	*2*(E2a) + *4*(A3d)	
G-60.05	*60.05.3*	1605	*2*(B2d-f)	
G-60.10	*60.10.4*	697	*2*(D5b)	
G-60.21	*60.21.2*	1290	*2*(D5b)	
G-60.24	*60.24.3*	1200	*2*(D4e-f)	
G-60.26	*60.26.1*	840	*2*(Bli)	*3*(C3c) + *5*(D1f)
G-60.26	*60.26.2*	793	*2*(G3a-b)	*5*(G1a)
G-60.29	*60.29.1*	1500	*2*(D3g)	
G-70.03	*70.03.4*	405	*2*(F5d)	
G-70.09	*70.09.1*	1320	*2*(C7e)	
G-70.09	*70.09.5*	450	*2*(G1b)	
G-70.10	*70.10.1*	1200	*2*(A1a)	
G-70.10	*70.10.5*	550	*2*(A2c) + *4*(G4e)	
G-70.14	*70.14.3*	1005	*2*(B2a) + *2*(G5h)	
G-70.16	*70.16.2*	1320	*2*(G5b)	
G-70.18	*70.18.1*	2000	*2*(F1d)	
G-70.19	*70.19.1*	1165	*2*(B3f)	
G-70.20	*70.20.2*	1150	*2*(B2c)	
G-70.20	*70.20.4*	650	*2*(C3e)	
G-80.07	*80.07.1*	2000	*2*(G3a-b)	*2*(D5a)
G-80.07	*80.07.2*	1085	*2*(E5e)	
G-80.07	*80.07.3*	937	*2*(E5a)	
G-80.09	*80.09.1*	560	*2*(A4a)	
G-80.13	*80.13.2*	1035	*2*(B2c)	
G-80.17	*80.17.1*	1450	*2*(A4e)	*2*(B2c)
G-80.20	*80.20.2*	500	*2*(A4d) + *3*(B4d)	
OPA-14	*OPA-14.1*	450	*2*(D2d)	
OPA-14	*OPA-14.2*	400	*2*(D2d)	
G-50.28	*50.28.5*	269	*3*(G4e)	
G-60.02	*60.02.2*	555	*3*(D5d)	
G-60.03	*60.03.3*	1530	*3*(C2c)	
G-60.03	*60.03.4*	1200	*3*(G5d) + *3*(centromere)	
G-60.05	*60.05.5*	685	*3*(G2d)	*4*(A4c)
G-60.09	*60.09.3*	757	*3*(A1e)	
G-60.10	*60.10.5*	355	*3*(E4c)	
G-60.24	*60.24.2*	1400	*3*(C5c) + *3*(D4b)	
G-60.26	*60.26.3*	424	*3*(C3c)	
G-70.03	*70.03.1*	575	*3*(F4a)	
G-80.10	*80.10.3*	925	*3*(A1b)	*3*(G2a)
G-80.12	*80.12.1*	1200	*3*(E1f-g)	
G-80.16	*80.16.1*	1760	*3*(A2d)	
G-50.22	*50.22.2*	557	*4*(E1c)	
G-50.25	*50.25.2*	627	*4*(A1g)	
G-50.28	*50.28.3*	885	*4*(A2e)	
G-60.10	*60.10.1*	1535	*4*(C1b)	
G-60.29	*60.29.2*	1115	*4*(A2g-h)	*5*(G1a)
G-70.01	*70.01.2*	812	*4*(G1f)	

(*continued*)

TABLE 2

(Continued)

Primer	RAPD	Size (bp)	Hybridization signal	
			Primary	Secondary
G-70.01	*70.01.3*	777	*4*(G1f)	
G-70.04	*70.04.1*	703	*4*(E2d)	
G-70.08	*70.08.1*	1092	*4*(G1g)	
G-70.09	*70.09.2*	1200	*4*(G1e) + *5*(G3e)	
G-70.09	*70.09.3*	1005	*4*(E4g) + *5*(C2a)	
G-70.09	*70.09.4*	898	*4*(E4g)	
G-70.09	*70.09.6*	400	*4*(C3g)	
G-70.12	*70.12.1*	925	*4*(C2d) + *4*(D2b)	
G-70.12	*70.12.3*	590	*4*(E4b)	
G-70.12	*70.12.4*	555	*4*(E4b)	
G-70.16	*70.16.3*	1130	*4*(G1d)	
G-80.09	*80.09.4*	453	*4*(D4a)	
G-80.09	*80.09.5*	449	*4*(D2a)	
G-80.10	*80.10.1*	2000	*4*(E1b)	
G-80.10	*80.10.2*	1100	*4*(E1d)	
G-80.14	*80.14.2*	1030	*4*(C2a) + *4*(G1b-c)	
G-80.16	*80.16.3*	848	*4*(F3c)	
G-80.16	*80.16.4*	616	*4*(D2a)	*2*(E6e)
G-80.16	*80.16.5*	543	*4*(E2g)	
G-80.19	*80.19.1*	2500	*4*(G4a)	
G-50.28	*50.28.1*	1590	*5*(G2f)	*3*(B4d)
G-60.03	*60.03.6*	905	*5*(D1f)	
G-60.27	*60.27.1*	590	*5*(G4a)	
G-60.29	*60.29.3*	1100	*5*(G1b)	
G-70.13	*70.13.3*	479	*5*(B1a)	
G-70.13	*70.13.4*	421	*5*(B1a)	
G-80.07	*80.07.4*	775	*5*(G4b)	
G-80.12	*80.12.3*	610	*5*(G2c)	
G-80.19	*80.19.3*	870	*5*(B3c)	
G-50.21	*50.21.1*	650	*2*(E3b) + *2*(G5) + *4*(A4f) + 5(F1d) + *3*(B5d) + *3*(G4)	
G-50.25	*50.25.1*	860	*X*(A1f) + *3*(C4c) + *2*(D3a-g) + *2*(E2a) + *3*(G2) *4*(C3c) + *5*(A3f)	
G-50.28	*50.28.2*	905	*4*(G3) + *X*, *2*, *3*, *4*, *5*(centromeres)	
G-60.05	*60.05.4*	1530	*2*(G1e) + *2*(B3e) + *2*(centromere)	
G-60.05	*60.05.6*	600	*3*(F2b-c) + *3*(F2c-d) + *4*(A2)	
G-60.06	*60.06.1*	1365	*X*, *2*, *3*, *4*, *5*(centromeres)	
G-70.03	*70.03.3*	495	*X*(B2g) + *2*(E4c) + *2*(G1e)	
G-70.10	*70.10.2*	840	*2*(G3b) + *4*(B2d) + *2*, *4*, *5*(centromeres)	
G-70.14	*70.14.1*	1150	*2*(G3b) + *4*(B1d) + *5*(G3c-d) + *2*(centromere)	
G-70.14	*70.14.2*	1130	*2*(G2i-j) + *3*(D4b) + *4*(A5a)	
G-70.14	*70.14.4*	950	*2*(B1k) + *2*(D3a-b) + *2*(centromere)	
G-70.16	*70.16.1*	1630	*X*(F3b) + *X*(G2f) *X*, *3*, *5*(centromeres)	
G-80.12	*80.12.2*	650	*4*(B1b) + *5*(E3d) + *5*(G2a)	
G-80.14	*80.14.3*	785	*2*(D5g-h) + *3*(A2b) + *4*(G1f)	
G-80.17	*80.17.2*	800	*2*(B2b) + *2*(G1a-f) + *5*(E4a-e)	
G-80.20	*80.20.1*	755	*X*(centromere) + *2*(B3) + *4*(D1)	
G-80.20	*80.20.3*	450	*2*(A4a) + *3*(A1c) + *3*(B1) + *5*(A3d)	
OPA-7	*OPA-7.1*	1200	*3*(D/E) + *4*(F3d) + *5*(C2d)	
OPA-7	*OPA-7.2*	800	*2*(E4/5) + *4*(A5b) + *4*(F1e-f)	
OPA-17	*OPA-17.2*	1400	>5 positions	
G-70.03	*70.03.2*	585	~15 positions	

Chromosome and site (in parentheses) refer to the cytological map of *D. repleta* (WHARTON 1942). Those 39 RAPD markers for which STSs were obtained are underlined (see Table 3).

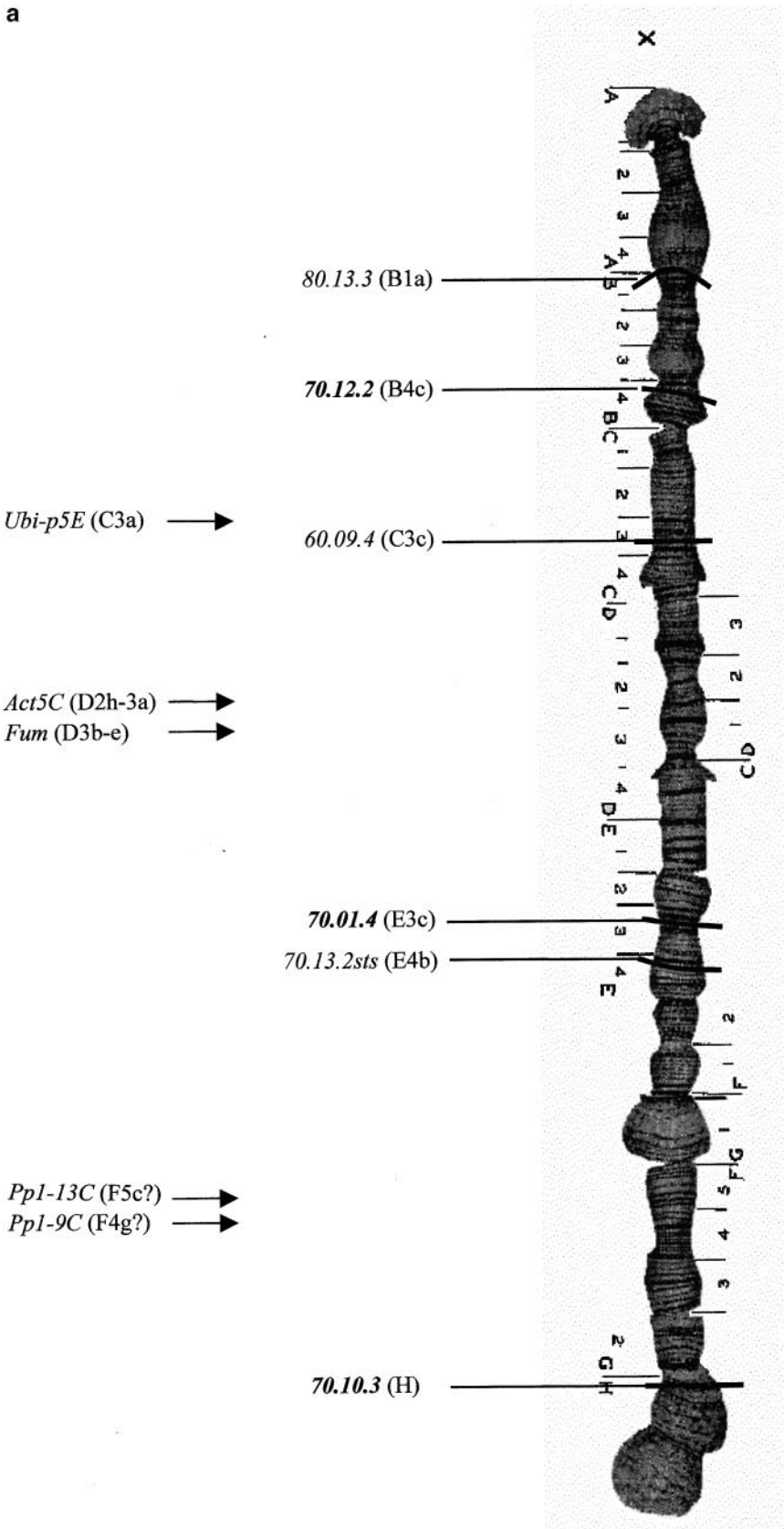

FIGURE 2.—Blueprints of the standard chromosome arrangements of *D. buzzatii* indicating the cytological localizations of the 73 RAPDs with a single primary signal, together with the 9 RAPDs that produced two primary signals, each on different chromosomes (indicated in boldface type), as inferred from the *in situ* hybridizations. (a) Chromosome X, (b) chromosome *2*, (c) chromosome *3*, (d) chromosome *4*, (e) chromosome *5*. The standard arrangements are cut-and-paste reconstructions of the *D. repleta* map (WHARTON 1942) according to the sequence of inversions proposed for their respective phylogenies (RUIZ and WASSERMAN 1993). The relative order of those markers that hybridized on the same band is not known for certain. On the basis of information in Table 4, *50.25.2sts* on *4*(A1g) likely marks the homologous to gene *kls*, and *80.12.3sts* on *5*(G2c) the homologous to gene *shot*, both in *D. melanogaster*. The breakpoints of the polymorphic inversions on the second (*2j*, $2jz^3$, $2jq^7$) and fourth (*4s*) chromosomes are also shown. To recover the chromosomal segments included in inversions $2z^3$ and $2q^7$, segment *2j* first must be inverted. The question mark indicates that the distal breakpoint of inversion $2z^3$ is not the same as that for inversion *2j* (see Figure 3). The positions of genes previously mapped in *D. buzzatii* are indicated by arrows (for *Fum* on chromosome *X* and *Pgm* on chromosome *4*, see NAVEIRA *et al.* 1986; for *Adh* on chromosome *3*, see LABRADOR *et al.* 1990; for the rest of the genes, see RANZ *et al.* 1997, 1999).

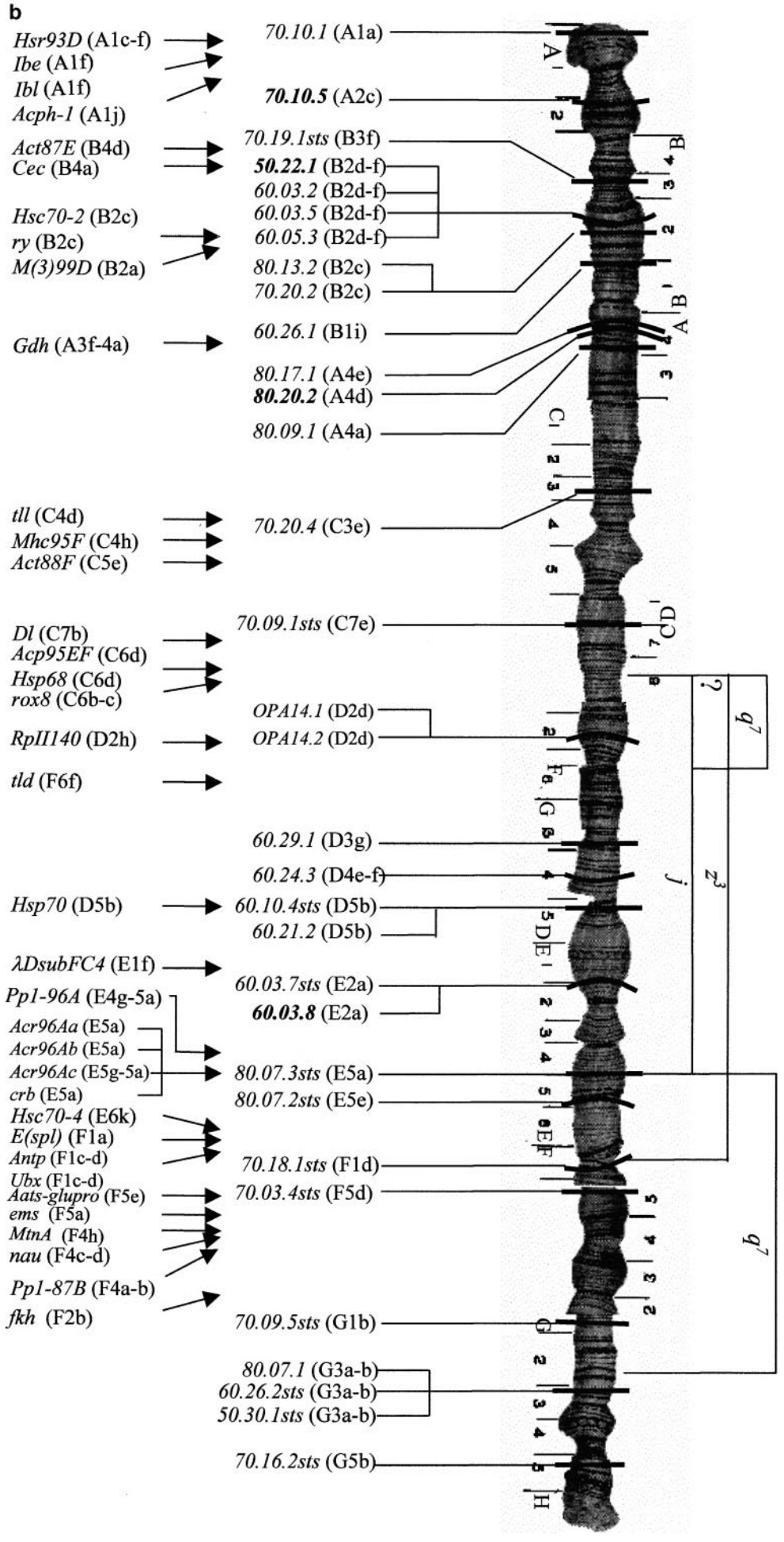

FIGURE 2.—*Continued.*

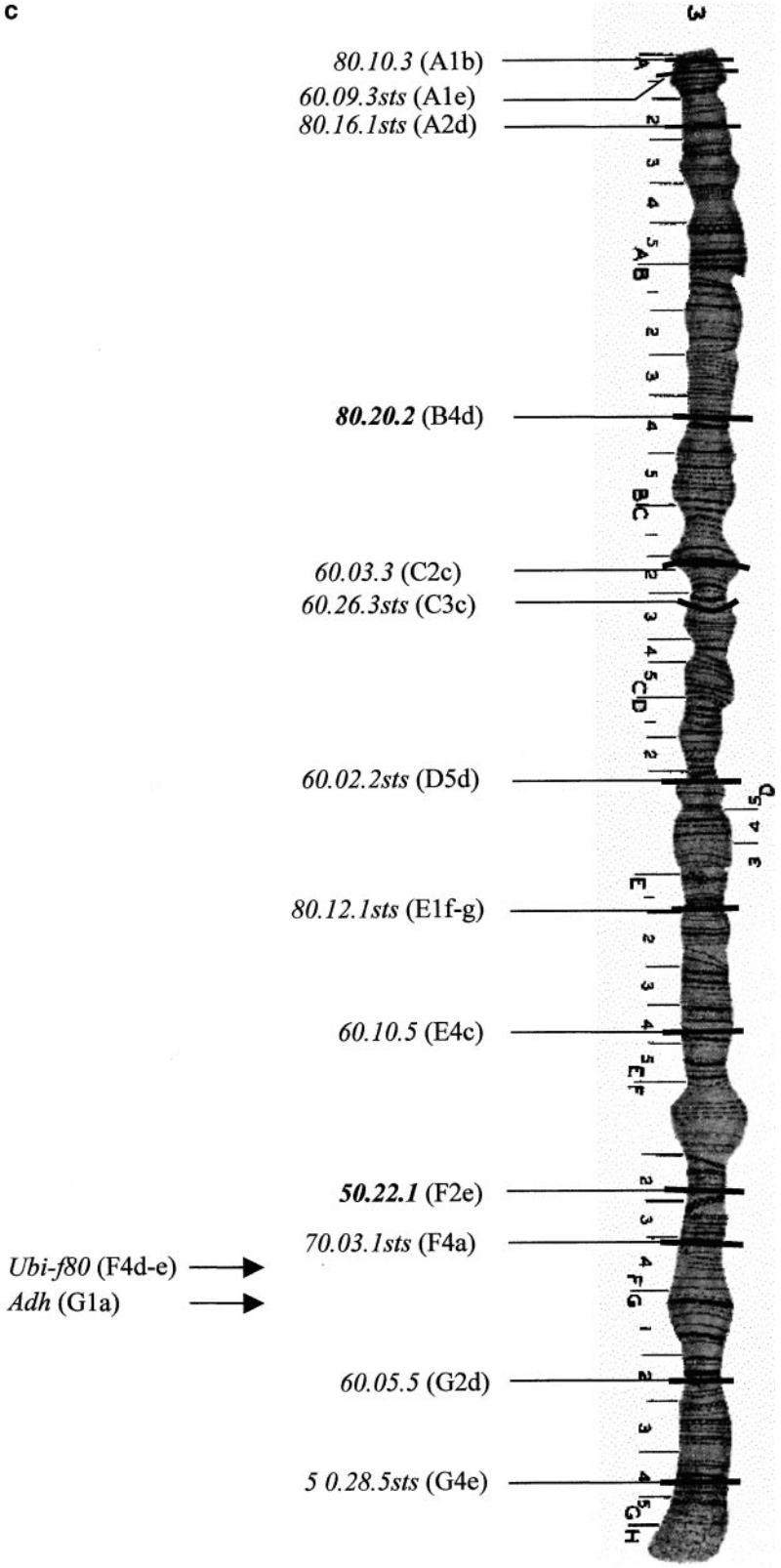

FIGURE 2.—*Continued.*

(RUIZ *et al.* 1984). This RAPD was also used as probe for *in situ* hybridization on a *D. buzzatii* strain fixed for $2jz^3$ gene arrangement. Figure 3 shows the hybridization signals, and it is clear that *70.18.1* is not included within the inverted fragment. However, some discrepancies were apparent when comparing the position of the hybridization signal with the putative distal chromosome structures that should be observed. Thus, if we assume

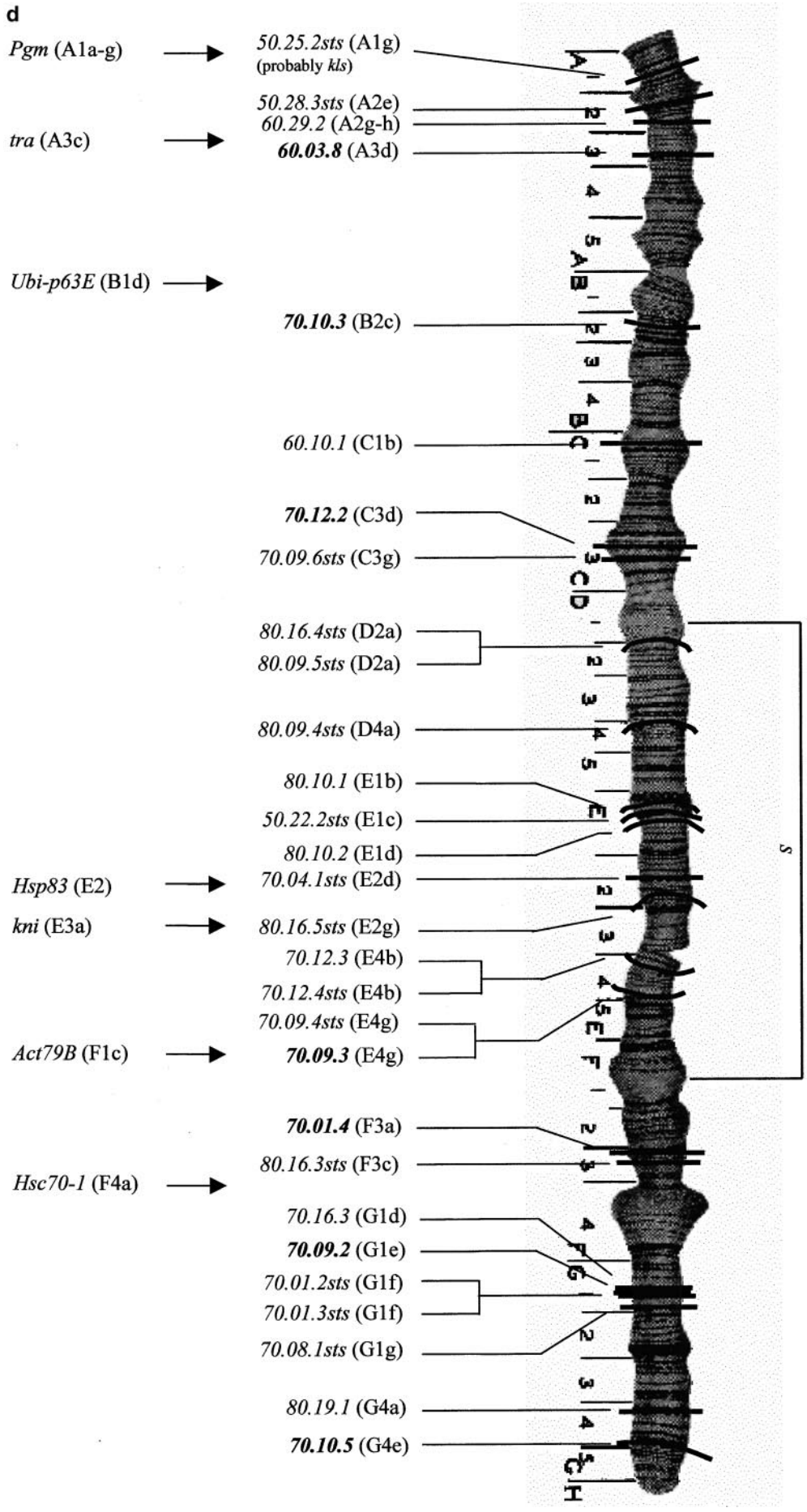

FIGURE 2.—*Continued.*

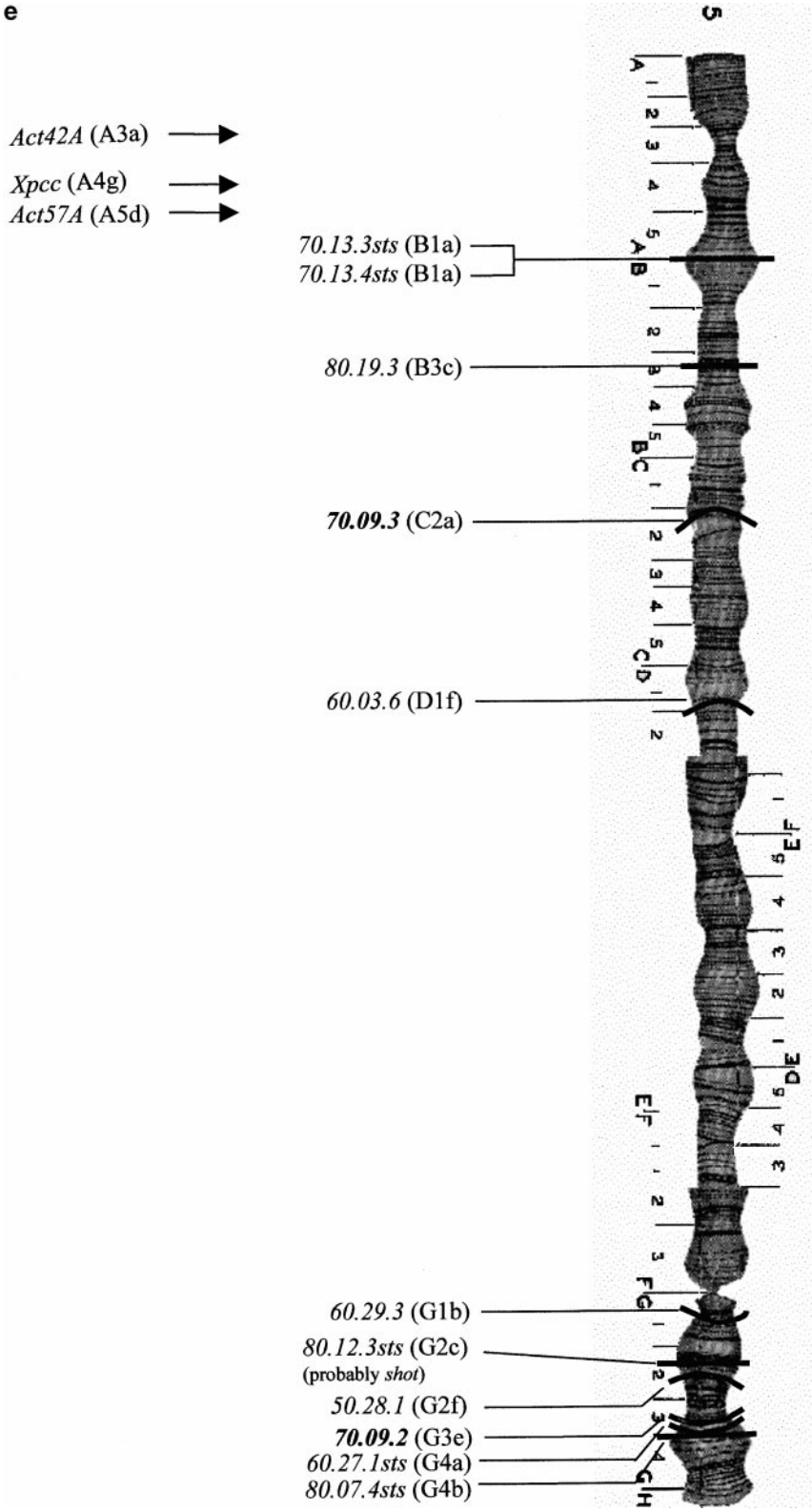

FIGURE 2.—*Continued.*

that the distal breakpoints of *2j* and *2z*^{3} were exactly the same on 2(C6c) (see RUIZ *et al.* 1984), the segment *2*(E4) should lie just after (proximal → distal direction) the hybridization signal and this was not the case. The most likely explanation is that the distal breakpoint of *2z*^{3} is indeed more proximal than that for *2j*, somewhere around *2*(E4b-c).

A conspicuous feature from Figure 2, a–e, is that RAPDs are unevenly distributed among chromosomes. From the putative homologies of *D. buzzatii* with the

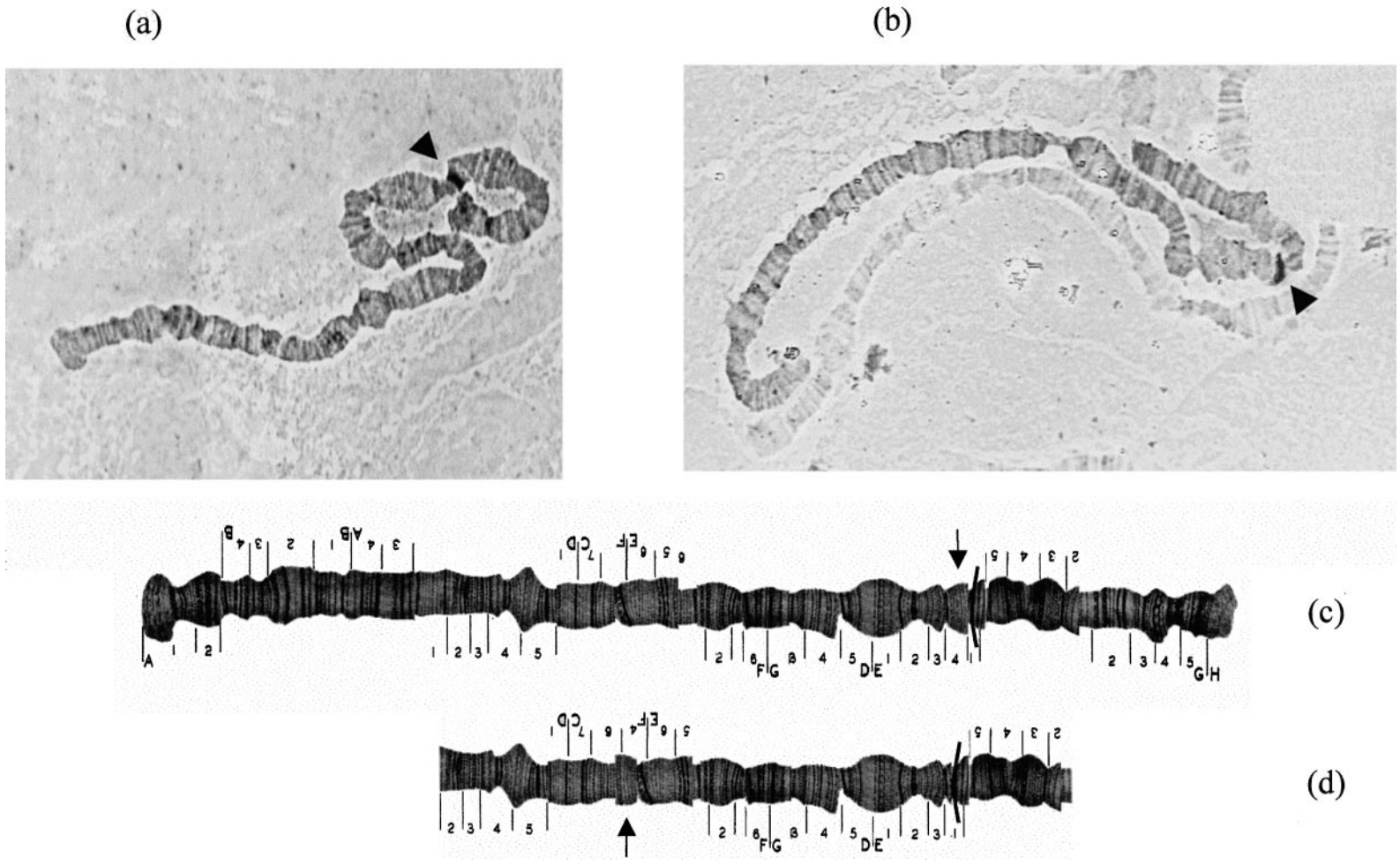

FIGURE 3.—*In situ* hybridization of RAPD *70.18.1* (arrowheads), which maps on *2*(F1d), using *D. buzzatii* strains fixed for (a) *2st* or (b) $2jz^3$ gene arrangements. It is clear that this marker is not included within the inverted segment $2z^3$. (c) The current consensus map for $2jz^3$ gene arrangement of *D. buzzatii*; the position of the hybridization signal is marked with a thick line. As previously indicated (Figure 2b), segment *2j* (from C6c to E5a; see RUIZ *et al.* 1984) must be inverted first to recover the chromosomal segment included in inversion $2z^3$ (from C6c to F1c). Note the position of segment E4 (indicated by arrows), which should be very close to the hybridization signal if the consensus map were correct. This is not observed in the *in situ* hybridization on $2jz^3$, and the most likely explanation is shown in d where it is assumed that the distal breakpoint of $2z^3$ is E4b-c.

Mueller/Sturtevant/Novitski chromosomal elements, and the percentage of total euchromatin assigned to each of these elements (*X-A*, 18%; *2-E*, 22.6%; *3-B*, 21.4%; *4-D*, 20.3%; *5-C*, 17.7%; see Wasserman 1982; Schafer *et al.* 1993), a higher-than-expected number of single-signal RAPDs were located on chromosomes *2* and *4* ($\chi^2_{(4)} = 22.0$; $P < 0.001$. The conclusion does not qualitatively change after correcting for the different number of *X* chromosomes in males).

Twenty-one RAPDs gave more than two primary signals on the salivary gland chromosomes, and in a number of cases they were located on the centromeres (Table 2). The presence of other copies of the same gene family, pseudogenes or DNA segments sharing a sequence homology, and/or transposable elements of diverse types are probably the reason to observe multiple signals.

Clustering of RAPDs: The extent of clustering of RAPD markers on chromosomes *2* and *4* (*i.e.*, those with a higher number of RAPDs) was investigated by means of a goodness-of-fit test (Sokal and Rohlf 1995) of the observed number of hybridization signals per chromosome section to that expected from a Poisson distribution. Chromosome *2* is divided into 38 sections (Wharton 1942) of ~600 kb each (assuming that *D. buzzatii* has ~2000 bands in the polytene chromosomes as *D. hydei* and ~50 kb per band; see Laird 1973; Hartl *et al.* 1994), and the distribution of signals (Figure 2b) was as follows: 12 sections with one signal, 4 with two, 2 with three, and 1 with six ($G_{(\text{Williams' correction})} = 9.41$; $P = 0.094$). The previous values could overestimate the degree of clustering because RAPDs *60.03.2* and *60.03.5* [section *2*(B2)], RAPDs *OPA14.1* and *OPA14.2* [section *2*(D2)], and RAPDs *60.03.7* and *60.03.8* [section *2*(E2)] might represent the same loci. Chromosome *4* is divided into 32 sections (Wharton 1942), and the distribution of signals (Figure 2d) was the following: 4 sections with one signal, 5 with two, 2 with three, 1 with four, and 1 with five ($G_{(\text{Williams' correction})} = 13.18$; $P = 0.010$). As for chromosome *2*, this distribution of signals could overestimate the degree of clustering because RAPDs *70.12.3* and *70.12.4* [section *4*(E4)] and RAPDs *70.01.2* and *70.01.3* [section *4*(G1)] might represent the same loci.

To see whether or not that was indeed the case, we derived STSs for RAPDs *70.01.2* and *70.01.3* and compared their sequences. They could be unambiguously aligned and matched almost perfectly with a big gap from nucleotides 494 to 511 due to a higher number of GT repeats in *70.01.2sts* (see Table 3 for their partial sequence information). After counting those two sets of RAPDs as a single marker, $G_{(\text{Williams' correction})} = 10.63$ ($P = 0.014$). Therefore, RAPDs are not randomly distributed along chromosome *4*, and there seems to be a higher-than-expected number of hybridization signals in the central part.

Similarly, we derived STSs for RAPDs *70.13.3* and *70.13.4* on chromosome *5*(B1a) and compared their sequences. They could be unambiguously aligned but there is a big indel of 74 nucleotides and a significant number of mismatches. This suggests that they could represent two closely related loci, but for the time being we cannot discard the possibility of a length polymorphism. To summarize, it is not clear whether or not all the RAPDs that were obtained from an identical decameric primer and happen to hybridize on the same chromosome band necessarily characterize the same locus. On the other hand, dissimilar RAPDs (*i.e.*, those obtained with different decameric primers) that map to the same location likely mark different loci (*cf. 60.26.2sts* and *50.30.1sts* on chromosome *2* and *80.16.4sts* and *80.09.5sts* on chromosome *4*).

Sequence analyses of RAPD markers: A total of 39 cytologically mapped RAPD markers were gel purified, reamplified by PCR, and cloned using T vectors. The clones were subjected to partial DNA sequence analysis from both ends and thus were converted to STSs, which are valuable markers for physical map construction and can also reveal previously undescribed potential genes (Louis *et al.* 1997). In most cases the total base pair length of the clones was sequenced, representing an aggregate sequence of 28,431 bp (27,654 bp after excluding *70.01.3sts*; see above). STS landmarks were designated by adding the suffix "*sts*" to the name of the original RAPD marker. Table 3 lists these STSs and presents the terminal 30 bp from each end. In all cases but three (see the slight variation in primer sequences reported as a footnote in Table 3), the decamer oligonucleotide that was used to generate the RAPD was present at each terminus as expected.

STSs allow us a rough approximation of the variation in nucleotide composition over the different chromosomes of *D. buzzatii*. Thus, overall G + C content is 41.18% (compared to 42.86% for *D. melanogaster* and 40.82% for the distant relative *D. virilis*; both values estimated from the buoyant densities reported in Gall *et al.* 1971), and the corresponding figures for the autosomes are the following: 36.05% for chromosome *5* (aggregate sequence of 2252 bp), 38.87% for chromosome *3* (aggregate sequence of 4809 bp), 40.54% for chromosome 4 (aggregate sequence of 9383 bp), and 43.52% for chromosome *2* (aggregate sequence of 10,219 bp). Assuming that STSs are representative of the whole genome, these figures would tentatively suggest that chromosome *2* is relatively rich in coding regions (see Li 1997).

All STS sequences were checked against both nucleic acid and protein databases for potential matches. We were particularly interested in those STSs (*80.07.3sts* and *70.18.1sts*) derived from the two RAPDs that map near second chromosome paracentric inversion breakpoints (Figure 2b). Thus, the proximal breakpoint of inversion *2j* lies between the *nAcR*β*-96A* and *Pp1*α*-96A* genes, has been recently cloned and sequenced, and contains large insertions corresponding to a transpos-

TABLE 3

Partial sequence information of RAPD STSs from *D. buzzatii*

STS	Accession no.	Sequence of 5′ and 3′ ends		
50.22.2sts	AF287294	CGAAACAGTCAACAGTCCACATAGAAGGCT-	...497...	-TCCACTACAGAGGCACTATCGACTGTTTCG
50.25.2sts	AF287295	ATCTGAGGAGCATCACAGCAGCATCTCGGA-	...567...	-GCAACCAATGCAAATCTCTCCTCCTCAGAT
50.28.3sts	AF287296	GATTGCGA[a]TTCATACGGACAGACGGACGG-	...770...	-GGTGGGTTGAGCTATACCTTGAACGCAATC
50.28.5sts	AF288312	GATTGCGTTCATACGGACAGACGGACAGAC-	...209...	-AAGATTTGTTTTGCTGCTTGGAACGCAATC
50.30.1sts	AF288313	TGCTGTGAACCAGAATTTCCATTTATGATG-	...491...	-TCTTCATCCTCCTGTTTACGGTTCACAGCA
60.02.2sts	AF288314	GTCCTACTCGCCACGAGCACAAGTCGAAAG-	...495...	-ATTAGGACAAAACAGGACACCGAGTAGGC[a]C
60.03.7sts	AF288315	CTACACAGGCTCTCGGGAATTTAGAAAATC-	...496...	-TCTCTGAATCGTTTACATTTGCCTGTGTAG
60.09.3sts	AF288316	CGTCGTTACCCAGATGCTAAAGTTTTTGGT-	...697...	-CACATGGCAGTACGGGTGCAGGTAACGACG
60.10.4sts	AF288317	GCAGACTGAGGTAAACGCAATGGCTGCGAA-	...637...	-ATCATAAATTGCTTCTAAGTCTCAGTCTGC
60.26.2sts	AF288344	GCAGCTCATGCAGAATAGACGTGGGACAAA-	410..(∼60)..263	-TCTTCATCATCATCGTGTAACATGAGCTGC
60.26.3sts	AF288318	GCAGCTCATGGAAAATAAATGTGAAAGTCT-	...364...	-ATTGATGAAACTTCTTTAGACATGAGCTGC
70.01.2sts	AF288336	CATCCCGAACCTTGAATGCCAAGCCAAAGC-	...752...	-TTGTTCGTTGTTCGTTGTCTGTTCGGGATG
70.01.3sts	AF288337	CATCCCGAACCTTGAATGCCAAGCCAAAGC-	...717...	-TTGTTCGTTGTTCGTTGTCTGTTCGGGATG
70.03.1sts	AF288319	ACGGTGCCTGCCACGTCGGCGGATTCATTG-	...809...	-ACGACCTTCACCTGCCATACCCAGGCACCG[a]
70.03.4sts	AF288320	ACGGTGCCTGGGGGAGGGCAAAGTGCTGAA-	...345...	-CATTCCATGGAACACGTTTCGCAGGCACCG
70.04.1sts	AF288321	CGCATTCCGCAGCAGATACAAAGACGAGCC-	...643...	-TTTTTGACTTGGTTTTTCGTGCGGAATGCG
70.08.1sts	AF288322	CTGTACCCCCCTCTCAGCCATGCGCAATGT-	...1032...	-ACGTGCTTATGTATTTGGCCGGGGGTACAG
70.09.1sts	AF288346	TGCAGCACCGTGGTAACGACGCACACGCGC-	626..(∼39)..595	-GTGGATCGCTGTTCTGAGTGCGGTGCTGCA
70.09.4sts	AF288323	TGCAGCACCGCGACATACGGGTTCTCAACA-	...838...	-CCCAGGTCATTCATAATGGTCGGTGCTGCA
70.09.5sts	AF288324	TGCAGCACCGAAAACTCCCAAGAACTTTAT-	...390...	-TTAATTTGCCATTTTGTAGCCGGTGCTGCA
70.09.6sts	AF288325	TGCAGCACCGATGCCAATGGCAACAGCAAT-	...340...	-TGCCGATGCGGGTGTGAGTGCGGTGCTGCA
70.12.4sts	AF288326	GGCCTACTCGCATTAGACTAATCCATTAAT-	...495...	-AACCCAACCCAACCACCCGTCGAGTAGGCC
70.13.2sts	AF288348	GTGTAGGGCGCTATTTATGTTCGCTAGTGA-	344..(∼374).587	-CGGCAGCTGCGTTCTCCGCACGCCCTACAC
70.13.3sts	AF288327	GTGTAGGGCGAAGTAAAATGATTTAATGAA-	...419...	-GCATCATTGACACACCGTTGCGCCCTACAC
70.13.4sts	AF303455	GTGTAGGGCGCAACGGTGTGTCAATGATGC-	...361...	-ATTTATGATTCATTTTACTTCGCCCTACAC
70.16.2sts	AF288350	CAGGGGCATCCAAAAAAAAAATAAGCCACAA-	505..(∼137).618	-ATGCAATTGTTTATATTGAAGATGCCCCTG
70.18.1sts	AF288352	GGCCTTCAGGAGTATCAGGGTATCAGGAGT-	650..(∼639).651	-TGATTAATCGGCTTAAATTACCTGAAGGCC
70.19.1sts	AF288338	GCTCTCACCGCAGGGGATTGGCGGTAATTT-	472..(∼180).448	-TTTTGGACATGCCGGTGGTTCGGTGAGAGC
80.07.2sts	AF288328	GCACGCCGGAGGGTCTTATTTACATGGCCC-	..1025..	-ATGTGACCGCGTTTATTGCATCCGGCGTGC
80.07.3sts	AF288329	GCACGCCGGAGGGGAACAGCATACTGTTGT-	...877...	-ACGGGCTTGGGGTCTTGTGTTCCGGCGTGC
80.07.4sts	AF288330	GCACGCCGGACCAGTTGTTAAAAGAGAATC-	...715...	-CAGTGCAGTGCTCACACATATCCGGCGTGC
80.09.4sts	AF288331	GCACGGTGGGGCATGGTATGGAACTAAAAA-	...393...	-TTGCTCTTTTAGCTTGTTTGCCCACCGTGC
80.09.5sts	AF303456	GCACGGTGGGGCATGGTATGGAGCTAAAAA-	...389...	-CCTTGCTCTTTGGCTTGTTGCCCACCGTGC
80.12.1sts	AF288340	CGACGCGTGCAGAAATGAAGCAAAGTCTTC-	505..(∼40).587	-CGTATTTGCTCTTCGTCTTGGCACGCGTCG
80.12.3sts	AF288332	CGACGCGTGCCAAACTCTTGTGGAATAAGT-	...517...	-CAAACAGTTTGGCAAGCTCTGCACGCGTCG
80.16.1sts	AF288342	ACCGCCTCCCCACACAGAAACAACCAACAG-	412..(∼978).311	-GAGCTGGGGCTATGCGCAGTGGGAGGCGGT
80.16.3sts	AF288333	ACCGCCTCCCCCAAATAGAAAGAAAAACAT-	...788...	-ACTATACCCCAAACTTCATTGGGAGGCGGT
80.16.4sts	AF288335	ACCGCCTCCCGTATCAATTCTCGCCTGCCA-	...556...	-AAAGCTGCATGTGTGTTTGGGGGAGGCGGT
80.16.5sts	AF288334	ACCGCCTCCCCCACAGTCAATATTAACCAA-	...483...	-AGAATTGTTAGCTACATTGTGGGAGGCGGT

The first 30 nucleotides (including the primer, underlined) are presented in each case, together with the length of the intervening DNA sequence between the sequences shown (those values in parentheses indicate the approximate length of the unknown intervening sequence). *70.01.2sts* and *70.01.3sts* on *4*(G1f) (Figure 3d) surely mark the same locus (see text for details).

[a] For *50.28.3sts* the expected sequence from the 5′ end based on the G-50.28 primer should be 5′-GATTGCGTTC-3′, for *60.02.2sts* the expected sequence from the 3′ end should be 5′-CGAGTAGGAC-3′, and for *70.03.4sts* the expected sequence from the 3′ end should be 5′-CAGGCACCGT-3′.

able element named *Galileo* (Cáceres *et al.* 1999). *80.07.3sts* was checked against both nucleic acid and protein databases and "hits" with an apparently unknown gene in Drosophila (see below).

As in the distant relative *D. virilis* (von Allmen *et al.* 1996), the genes *Antennapedia* (*Antp*) and *Ultrabithorax* (*Ubx*) in *D. buzzatii* seem to be together because they map on the same *2*(F1c-d) band (Ranz *et al.* 1997), close to the putative proximal breakpoint *2*(F1c) of inversion $2z^3$ (Ruiz *et al.* 1984). *70.18.1sts* maps on *2*(F1d) (Figure 2b) and, as described above, is not included within the inversion fragment (Figure 3). No significant hits with known genes were found in BLAST searches for *70.18.1sts*, and we do not know whether the relative positions of *Antp-Ubx-70.18.1sts* are still conserved in the $2jz^3$ gene arrangement.

Table 4 lists the 22 STSs that rendered significant "hits" in BLAST searches of the GenBank databases and also shows the protein alignments between conceptually translated STSs and the respective hits representing known genes. As expected, the significant hits were in most cases with protein sequences or genomic scaffolds from *D. melanogaster*, but in three instances (*70.09.4sts*, *80.07.3sts*, and *70.19.1sts*) the hits were with protein sequences from other taxa that have not yet been described in Drosophila. Interestingly, *70.09.4sts* and *80.07.3sts* show reasonably good alignments with their corresponding matches (see Table 4) and might have identified novel Drosophila putative genes.

Thirteen STSs hit with Drosophila sequences of known chromosomal location. From the alignments observed in Table 4 and the corresponding chromosomal homologies (see below), we conclude that *50.25.2sts* likely marks the homologous to gene *klarsicht* (*kls*) and *80.12.3sts* the homologous to gene *short stop* (*shot*); both genes were previously known and mapped in *D. melanogaster* (FlyBase Consortium 1999). An intriguing case is the hit of *80.12.1sts* with alcohol dehydrogenase (*Adh*) genes of *D. buzzatii* and the Tc1-like DNA transposable element of *D. virilis*. In many species of the *repleta* group (including *D. buzzatii*) the *Adh* region contains a pseudogene (*Adh-Ψ*) and two *Adh* functional genes (*Adh-2* and *Adh-1*), arranged 5′ to 3′, that have arisen by two independent duplication events (Menotti-Raymond *et al.* 1991; Yum *et al.* 1991; Sullivan *et al.* 1994). Alignment of *80.12.1sts* with the *D. buzzatii Adh* region (GenBank accession no. U65746) shows substantial matches between the intervening sequence of genes *Adh-2* and *Adh-1* (from nucleotides 5475 to 5580) and nucleotides 455...560 of *80.12.1sts*, and alignment with Tc1-like from *D. virilis* (GenBank accession no. U26938) shows substantial matches for nucleotides 276...312 of *80.12.1sts* with the inverted repeats of the element. *Adh* maps at the *3*(G1a) band in *D. buzzatii* (Labrador *et al.* 1990), while RAPD *80.12.1* maps at the *3*(E1f-g) band (Table 2, Figure 2c) and could reflect a transposon-mediated movement event.

An extensive reorganization within Mueller/Sturtevant/Novitski chromosome elements has occurred in Drosophila evolution, but chromosomal homologies have been generally conserved (Segarra and Aguadé 1992; Kress 1993; Hartl and Lozovskaya 1994; Segarra *et al.* 1995, 1996; Ranz *et al.* 1997; Vieira *et al.* 1997). This allowed us to check our hybridization signals with those reported for *D. melanogaster*, and in general there was a good agreement. Thus, *kls* maps in *D. melanogaster* on chromosome *3L*, very close to the telomere, and this agrees quite well with the position of RAPD *50.25.2* on chromosome *4* (Table 2 and Figure 2d). *shot* maps on chromosome *2R* and, accordingly, RAPD *80.12.3* maps on chromosome *5* in *D. buzzatii* (Table 2 and Figure 2e). In one case (*80.16.4sts*) the correspondence was with the secondary signal (chromosome *3R* in *D. melanogaster* and chromosome *2* in *D. buzzatii*; Table 2 and Figure 2b), and in three additional cases (*50.22.2sts*, *70.03.4sts*, and *80.07.2sts*) there was no correspondence with the cytological location reported for the genomic scaffolds in *D. melanogaster*. Similarities in sequences between different proteins are likely the cause for the lack of correspondence, which is clearly suggested by the two hits of *50.22.2sts* (Table 4).

Negative results: In spite of up to three attempts, 36 RAPDs (25%) did not produce any detectable hybridization signal on the polytene chromosomes (Table 5). We have obtained the DNA sequences from a sample of three of those RAPDs (*70.08.2*, *70.09.7*, and *70.14.5*, with an aggregate sequence of 1606 bp) to further investigate whether or not they present special features to prevent *in situ* hybridization. The three sequences have an overall G + C content (41.10%) very similar to the STSs, and no repetitive regions were detected. No significant hits were found when these sequences were checked against both nucleic acid and protein databases. However, the sequence *70.14.05* presents an ORF of 207 amino acids (data not shown), and several additional clues to suggest that this sequence is part of a coding region (compositional differences among codon positions relatively large and similar to the functional genes in *D. buzzatii*; *i.e.*, G + C highest in third position and lowest in second position).

A likely cause for the lack of hybridization signal can be an underreplication of those sequences during the formation of polytene chromosomes. This will be the case for all sequences within the α-heterochromatin and some sequences within the β-heterochromatin (Gall *et al.* 1971; Gall 1973; Glaser *et al.* 1997). However, no firm conclusion can be made on the available data and further work is in progress.

Conclusions and prospects: The present results help understand the observed differences in the distribution of genetic variation over chromosomes in species of the *repleta* group of Drosophila (Zouros 1976). Thus, enzyme heterozygosity is highest for chromosome *2*, but chromosomes *4* and *5* could not be adequately sepa-

TABLE 4

Sequences producing significant alignments with the *D. buzzatii* STSs in searches using the BLAST program

STS	Sequences with significant "hits"	E[i]
50.25.2sts [*4*(A1g][a]	*D. melanogaster* klarsicht protein. Chromosome *3L*(61C4) (AF157066)	2×10^{-61}
80.12.3sts [*5*(G2c)][b]	*D. melanogaster* P1 clone. Chromosome *2R*(50C6-8) (AC005977)	2×10^{-31}
	D. melanogaster groovin protein. Chromosome *2R*(50C3-4) (Y09430)	3×10^{-29}
60.26.2sts [*2*(G3a-b)][c]	*D. melanogaster* zinc finger motif protein (AF038865)	2×10^{-19}
60.09.3sts [*3*(A1e)][d]	*Methanococcus jannaschii* argininosuccinate synthase (U67494)	7×10^{-15}
	D. melanogaster argininosuccinate synthase (AE001574)	2×10^{-13}
70.09.4sts [*4*(E4g)][e]	*Escherichia coli* hexuronate transporter (P42609)	2×10^{-41}
80.07.3sts [*2*(E5a)][f]	*Caulobacter crescentus* CheB protein (AJ006687)	4×10^{-24}
70.09.1sts [*2*(C7e-d)][g]	*D. melanogaster* CG5237 gene product. Chromosome *3R* (AE003725)	3×10^{-34}
70.19.1sts [*2*(B3f)][h]	Hypothetical protein yuiI. *Bacillus subtilis* (B70013)	4×10^{-10}
80.16.4sts [*4*(D2a)]	*D. melanogaster* mRNA for CAKI protein. Chromosome *3R*(93F6-14) (X94264)	1×10^{-19}
80.16.5sts [*4*(E2g)]	*D. virilis* DNA for trithorax protein gene (Z50038)	5×10^{-8}
70.13.2sts [*X*(E4b)]	*D. melanogaster* clone BACH6115. Chromosome *X* (AL035245)	8×10^{-7}
80.12.1sts [*3*(E1f-g)]	*D. buzzatii* alcohol dehydrogenase. Chromosome *3*(G1a) (U65746)	2×10^{-8}
	D. virilis Tc1-like transposable element (U26938)	2×10^{-8}
70.16.2sts [*2*(G5b)]	*D. melanogaster* Micropia polyprotein (S02021)	7×10^{-32}
50.28.3sts [*4*(A2b)]	*D. melanogaster* ZAM retroelement (AJ000387)	4×10^{-41}
50.22.2sts [*4*(E1c)]	*D. melanogaster* CG6492 gene product. Chromosome *X* (AE003506)	1×10^{-18}
	D. melanogaster CG18340 gene product. Chromosome *2L* (AE003612)	1×10^{-14}
70.01.2sts [*4*(G1f)]	*D. melanogaster* genomic scaffold. Chromosome *3L* (AE003597)	5×10^{-7}
70.01.3sts [*4*(G1f)]	*D. melanogaster* genomic scaffold. Chromosome *3L* (AE003597)	3×10^{-11}
70.03.4sts [*2*(F5d)]	*D. melanogaster* genomic scaffold. Chromosome *3L* (AE003558)	7×10^{-25}
70.08.1sts [*4*(G1g)]	*D. melanogaster* genomic scaffold. Chromosome *3L* (AE003546)	7×10^{-16}
70.09.6sts [*4*(C3g)]	*D. melanogaster* genomic scaffold. Chromosome *3L* (AE003541)	6×10^{-11}
70.18.1sts [*2*(F1d)]	*D. melanogaster* genomic scaffold. Chromosome *3R* (AE003671)	7×10^{-12}
80.07.2sts [*2*(E5e)]	*D. melanogaster* CG1753 gene product. Chromosome *X* (AE003569)	1×10^{-104}

Access number is given in parentheses. Cytological locations of STSs are from Table 2, and those for *D. melanogaster* hits were obtained from FlyBase (http://astorg.u.strasbg.fr:7081). *70.01.2sts* and *70.01.3sts* are not independent (see text for details). Protein alignments between conceptually translated STSs and hits representing known *D. melanogaster* genes and/or presumably undescribed genes in Drosophila are given as footnotes from a to h. The numbers flanking the top lines indicate nucleotide positions of the amino acid residues in the STSs; numbers flanking the bottom lines indicate amino acid positions in the known protein. Percent similarity and identity are also indicated.

[a] *50.25.2sts* × klarsicht protein (*D. melanogaster*) (sim: 92%; iden: 89%)

```
1    SEEHHSSISETAWDNYQEKYNSENYSEGLDSDAARRLLEFGDDYRFDLCSSSNFIDSQSD 180
     :|:||||||||||||||||||||||||||| |||||||||||||||       ||||||||
1418 TEDHHSSISETAWDNYQEKYNSENYSEGFDSDAARRLLEFGDDYR-------NFIDSQSD 1470

181  CCSSLSAANNLDSFSPPRMDSLQQHELKALHINQGTISSSVDHARRQRALELQYERRRKT 360
     ||||||||||||||||||||||||:||||:||||| ||||||||||||||||||||||||
1471 CCSSLSAANNLDSFSPPRMDSLQKHELKSLHINQDTITSSVDHARRQRALELQYERRRKT 1530

361  LEVRRKSCQ 387
     |||||||||
1531 LEVRRKSCQ 1539
```

[b] *80.12.3sts* × groovin protein (*D. melanogaster*) (sim: 97%; iden: 91%)

```
6    RAKLLWNKWRDVWMLSWERQRLLHEHLMYLKDVERARNFSWDDWRKR 146
     |||||| |||||||||||||||::|||||||||||||||||||||||
3668 RAKLLWTKWRDVWMLSWERQRLLNDHLLYLKDVERARNFSWDDWRKR 3714

212  FLKYMNHKKSRLTDLFRKMDKDNNGMIPR 298
     |||||||||||||||||||||||||||||
3715 FLKYMNHKKSRLTDLFRKMDKDNNGMIPR 3743
```

[c] *60.26.2sts* × zinc-finger motif protein (*D. melanogaster*) (sim: 51%; iden: 33%)

```
408 CTQCAHGFTQHANLERHSCSGSEAASADPCSRHRCSHCGKCMQSASSLIMHLRLHSGERP 229
    |  |   :|    |: |  :  |         |:|  | |   :  :|  |:: |:||||
231 CPLCPKAYTHGPTLKSHMHTHDEEKG------HKCPQCDKTFYTRGNLRAHIQRHTGERP 284

228 FACDACPKTFKTNGGLVTHQKRHLKLLEYECEYCGKGFVESSNLRRHIASLHTQERPHICTVCQRTF 28
    : |  ||:||  | ||  | : | :   ::|| ||||||:: :|  |:  :|  :|   |  | ::|
285 YKCPDCPQTFAKNSGLKLHSRLHKEERPFKCELCGKGFVQNQHLITHL-RVHNGDRQFKCPDCDKSF 350
```

(*continued*)

TABLE 4

(Continued)

[d] *60.09.3sts* × argininosuccinate synthase-like (*D. melanogaster*) (sim: 54%; iden: 38%)

```
400 LTFSGGLDTSFCAMYLSKDKGYEVYTAVANTGGFSPEELKVIEEKAYKLGAKKHVTLDVT 579
    | :|||||||| | :    || |||   :|: |    |:    |:|| |:|||| :  ||
8   LAYSGGLDTS-CVLKWLLDKQYEVICVLADVG--QKEDFTAAEKKALKIGAKKVIVADVK 64

580 QEYYEKSIKYMIFGNVMRNGTYPISVSSERIFQAMAIINYAKEIGADAVAHGSTGAGND 756
    | : |  |   :   ::    | :  |  R   ::|::  |:| ||  :|||:|| |||
65  QSFVEDYIWPAVQMGLVYEERYLLGTSLARPCISVALMEVAREYGAKYLAHGATGKGND 123
```

[e] *70.09.4sts* × hexuronate transporter (*E. coli*) (sim: 66%; iden: 49%)

```
1   LAIIAAFVWFFFYKDPKDAKRLSDEERAYIENGQEKRLKSAKKEKTSVINILKQRNFWGI 60
    |: | |  |  ||| |:| | |:|||| || |||| : : :  :| ||  ||: | ||||
213 LSFIWAMAWLIFYKHPRDQKHLTDEERDYIINGQEAQHQVSTAKKMSVGQILRNRQFWGI 272

61  GLSRFLADPAWGTINFWVPIFFVETLHFSLKEIAMSVWLPFLMADLGCLASGFVAKYFND 120
     | ||||:|||| | |:|:|  :   |:|||||  |:| | |||||:  |::   |
273 ALPRFLAEPAWGTFNAWIPLFMFKVYGFNLKEIAMFAWMPMLFADLGCILGGYLPPLFQR 332

121 -RGVSLINSRRITFTIGAVLMTTIGLVSIVENPYVAVLMNDLG 162
      ||:|| ||::  |:|||||   |:: :  |||||:::  :|
333 WFGVNLIVSRKMVVTLGAVLMIGPGMIGLFTNPYVAIMLLCIG 375



138 VLMTTIGLVS--IVENPYVAV---LMNDLGITKEQYSWVVSAFQLAYTIGQPIMGFFIDT 192
    : : |:| |   :  |   |    || :| |: :|||:::|:  |||: ||: |: :|
52  IALVTLGTVLGYLTRNTVAAAAPTLMEELNISTQQYSYIIAAYSAAYTVMQPVAGYVLDV 111

193 IGLKLGFFICAIIWGLATMAHGLTGSWQGLAFMRGIMGFSEASAIPAGVKTATTWFPAKE 252
    :| |:|: : |::| :   |  | ||| |||  || :| :||: |||:| :: ||||||
112 LGTKIGYAMFAVLWAVFCGATALAGSWGGLAVARGAVGAAEAAMIPAGLKASSEWFPAKE 171

253 RGIATGVFNMGTSFGPMLAPPLIPWCIMFHSWK 285
    | || | ||:|:| | |:|||: | |: |||:
172 RSIAVGYFNVGSSIGAMIAPPLVVWAIVMHSWQ 204
```

[f] *80.07.3sts* × cheB protein (*C. crescentus*) (sim: 73%; iden: 64%)

```
7   GGEQHTVVERYADGLRTRLVKAPPVNGHSPSVDVLFDSVAAQVGPNALGILLTGMGQDGA 186
    ||  |  | | | ||| |||   ||:|| |||||||:|||  ||  |:|::|||||:|||
229 GGATHLEVVRSA-GLRCRLVAGDPVSGHRPSVDVLFNSVAHAVGDKAVGVILTGMGRDGA 287

187 KGLLAMRQAGAMTIAQDHDSSVVYGMPRVAAE 282
    :||| ||:||| |: ||  | |||||| | |
288 QGLLTMRKAGAKTLGQDEASCVVYGMPRSAFE 319
```

[g] *70.09.1sts* × CG5237 gene product (*D. melanogaster*) (sim: 52%; iden: 46%)

```
7    TVVTTHTRHSLQEGVRMIVTPLVGSETTETAIISPPVDVHRAVTVRNKSLENAAASTSKM 186
     |:|||||||||||||||||||||                        |||||||||||
1898 TLVTTHTRHSLQEGVRMIVTPL--------------------------SLENAAASTSKM 1931

187  FAAIATNHLKALGALQDLPTA----------SGSKPXXXXXXXXXXXXXATATLASSIA- 333
     |||||||||||||||||:| |          |||:              |    :|| |
1932 FAAIATNHLKALGALQDMPAAVERKAASSSGSGSRSANGSGNGSGGSAPAAIQASSSAAA 1991

334  -KSIGRHKTIVECXXXXXXXVDGSRQKKSQTK-VAETHRQ-GIWFAGLAAV*DERNAQS 504
      | |||||||||||         | |||||||| :  | :  |   : |: :   | :
1992 SKPIGRHKTIVECSAGNSSSSADDSRQKKSQTKSLRRTDKNYGSPDSPLSKMSVMPNPRD 2051

505  M**ARMRVCCHLPRVFPALEIPTPERTVAHWTQGTGATFVERMRIDLICP 654
          |:     |:   |||||||| :   || | || |||:|  |  |
2052 EMDESMQ-SLPPPKSIAALEIPTPERLLPIGTQDTVATLVERVRDGLNLP 2100
```

(*continued*)

TABLE 4

(Continued)

[h] *70.19.1sts* × hypothetical protein yuiI (*B. subtilis*) (sim: 64%; iden: 52%)

```
208 GGRSGSFYQFIAACVKPWIEAHYPVNADQQTLAGHSHGGHFVLYTLFNHPDAFQNYLAAS 387
    ||  | |::||   :|| ||  | ::  :||: ||| || |||  |   ||||| |:| |
154 GGAEG-FFRFIEEDLKPEIERDYQIDKKRQTIFGHSLGGLFVLQVLLTKPDAFQTYIAGS 212

388 PSI 396
    |||
213 PSI 215

38  LYLLDANSQFSVVTER-----NNRKRDGDI--LYIGIGYQDGVDILKARTRDYTVPSGE- 193
    :|||||||  |  :||         :: | |  : :||||:       || ||:|:|: :
80  IYLLDANSVFGTMTEAVRIQGRRPEKTGVIPAVIVGIGYETAEPFSSARHRDFTMPTAQS 139

194 --------KEFSEGGGAAAFINLLPHAL 253
            :|: | |||  |   :   |
140 KLPERPDGREWPEHGGAEGFFRFIEEDL 167
```

[i] Indicates the number of hits one can "expect" by chance when searching a database of a particular size.

rated and were treated as a unity. The apportioning of RAPDs observed here certainly suggests that average variability levels on the autosomes of *D. buzzatii* are $2 \geq 4 > 3 > 5$, contrary to the observed distribution of spontaneous visible markers that placed chromosome *4* as the least variable (Schafer *et al.* 1993).

The physical map of *D. buzzatii* now comprises 73 effectively unique RAPD markers (39 of these are STSs) and 53 genes whose cytological position is already known (Figure 2, a–e). On the other hand, the current genetic map is poorly developed and consists of three linkage groups (chromosomes *X*, *2*, and *5*) that include visible mutants and enzyme loci (Schafer *et al.* 1993). The RAPDs obtained here (along with those that gave secondary signals, those that gave hybridization signals on different chromosomes, and the 36 that did not give any signal) will be used as genetic markers to provide a link between the physical and more extensive linkage maps, also covering chromosomes *3* and *4*. In addition, they will help to increase the density of markers (including microsatellites) around specific genomic regions to search for quantitative trait loci of fitness-related traits such as body size (see Betrán *et al.* 1998). [A caveat: because the cytological maps of *D. buzzatii* are cut-and-paste reconstructions of the *D. repleta* map (see above), exact correspondence between the physical and the genetic maps for the relative positions of markers is expected, provided the proposed cytogenetic relationships between *D. repleta* and *D. buzzatii* are fully correct.]

M. Labrador and J. E. Quezada-Díaz were of great help during the initial steps of this work. We thank A. Leibowitz and J. E. Quezada-Díaz for their assistance in collecting the thousands of flies raised

TABLE 5

RAPDs that did not give any hybridization signal on the salivary gland chromosomes of *D. buzzatii*

Primer	RAPD	Size (bp)	Primer	RAPD	Size (bp)
G-70.01	*70.01.1*	1400	G-80.13	*80.13.4*	905
G-70.08	*70.08.2*	495	G-80.19	*80.19.2*	2300
G-70.09	*70.09.7*	375	G-60.02	*60.02.1*	585
G-70.10	*70.10.4*	570	G-60.05	*60.05.2*	2200
G-70.14	*70.14.5*	735	G-60.06	*60.06.2*	1180
G-70.15	*70.15.1*	735	G-60.06	*60.06.3*	820
G-70.17	*70.17.1*	705	G-60.09	*60.09.1*	1540
G-70.18	*70.18.2*	1100	G-60.09	*60.09.2*	895
G-70.19	*70.19.2*	1100	G-60.10	*60.10.2*	810
G-70.19	*70.19.3*	952	G-60.10	*60.10.3*	780
G-70.20	*70.20.1*	1630	G-60.24	*60.24.1*	1605
G-70.20	*70.20.3*	705	G.60.24	*60.24.4*	1155
G-70.20	*70.20.5*	610	G-60.24	*60.24.5*	1070
G-80.09	*80.09.2*	540	G-60.26	*60.26.4*	395
G-80.09	*80.09.3*	500	G-60.29	*60.29.4*	895
G-80.09	*80.09.6*	420	G-50.21	*50.21.2*	520
G-80.09	*80.09.7*	400	G-50.21	*50.21.3*	480
G-80.13	*80.13.1*	1340	G-50.28	*50.28.4*	755

from Opuntia rots, L. Alarcón and F. Rodríguez-Trelles for providing information on the sequence of *Xdh* in *D. buzzatii* before publishing, M. P. García-Guerreiro for helpful advice with *in situ* hybridizations, F. Rodríguez-Trelles for helpful discussion and careful reading of earlier drafts, and M. Peiró for technical assistance. One of us (H.L.) is very grateful to M. R. Goldsmith for providing a stimulating intellectual environment during his stay in the Department of Zoology, University of Rhode Island. Two anonymous referees and the communicating editor provided very helpful comments on the manuscript. H.L. was supported by a FP94-00215104 fellowship from the Ministerio de Educación y Ciencia (Spain). This work was supported by grants PB93/0843 and PB96-1136 from the Dirección General de Enseñanza Superior e Investigación Científica (DGESIC, Spain) to A.F. and grant SGR98 from the Direcció General de Recerca (Generalitat de Catalunya) to the GBE.

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Communicating editor: L. Partridge