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Analysis of *Drosophila* chromosome *4* using pulsed field gel electrophoresis

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Abstract. Previous estimates of the size of *Drosophila melanogaster* chromosome *4* have indicated that it is 1% to 4% of the genome or ~6 Mb. We have used pulsed field gel electrophoresis (PFGE) to separate megabase-sized molecules of *D. melanogaster* chromosomal DNA. Southern blots of these gels were probed with DNA fragments from the *cubitus interruptus* and *zfh-2* genes, which are located on chromosome *4*. They each identify the same-sized distinct band that migrates at approximately 5.2 Mb in DNA preparations from the Kc cell line. We interpret this band to be intact chromosome *4*. In DNA obtained from embryos of various *D. melanogaster* wild-type strains, this chromosome band showed strain-specific size variation that ranged from 4.5 to 5.2 Mb. The *D. melanogaster* chromosome *4* probes also identified a single, 2.4 Mb band in embryonic DNA from *Drosophila simulans*. We conclude that *D. simulans* chromosome *4* is substantially smaller than that of *D. melanogaster*, presumably owing to differences in the amount of heterochromatic DNA sequences. Our simple DNA preparation from embryos and PFGE conditions should permit preparative isolation of chromosome *4* DNA and will facilitate the molecular mapping of this chromosome.

Introduction

Chromosome *4* in *Drosophila melanogaster* is considerably smaller than the *X*, *Y*, second or third chromosomes. In standard metaphase spreads chromosome *4* appears only as a dot and is thought to be an acrocentric chromosome (Hochman 1973; Roberts 1972). In the much larger, more specialized polytene chromosomes of the salivary gland, chromosome *4* is a small segment that extends from the chromocenter with about 50 distinct bands, or 1% of the total number of polytene bands. The polytene banded region represents euchromatic DNA only and does not include the centromeric heterochromatin present on chromosome *4*. Size estimates obtained from ultraviolet absorbance measurements of metaphase chromosomes suggest that chromosome *4* is 3.5% of the genome (Hochman 1973; Rudkin as cited in Kavenoff and Zimm 1973), or ~6 Mb, based on a total genome size of 170 Mb. This size estimate of chromosome *4* is subject to uncertainty and has not yet been confirmed by other means.

Chromosome *4* is an atypical *Drosophila* chromosome. There is normally no crossing over on this chromosome in either sex. This has hampered genetic analysis of chromosome *4* genes. Unlike the other much larger chromosomes (the *Y* chromosome excepted), aneuploidy for chromosome *4* can be tolerated even though relatively mild phenotypic alterations do accompany such mutations (see Lindsley and Zimm 1992). Another unusual feature of this chromosome is that the euchromatic regions of chromosome *4* contain a higher frequency of repeated DNA sequences than the corresponding regions of other *Drosophila* chromosomes (Miklos et al. 1988). These repeated sequences appear not to be limited to the centromeric heterochromatin as on the other chromosomes but are dispersed in the euchromatic region of chromosome *4*. There have been at least 37 essential loci plus at least 6 other loci with recessive visible alleles mapped to chromosome *4*, and statistical estimates indicate that the total number of mutable loci is 70–80 (Hochman 1973).

The lack of recombinational mapping makes molecular mapping of this chromosome essential for *Drosophila* studies. Its small size and the presence of interspersed repeated sequences (as well as centromeric repeated sequences) also make chromosome *4* an ideal model for the study of normal higher eukaryotic chromosome structure. At the maximum estimated size of 6 Mb, this chromosome should be amenable to separation and isolation by pulsed field gel electrophoresis (PFGE). The use of low voltage and long switching times has permitted the separation of whole chromosomes from *Schizo-*

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saccharomyces pombe (3.5–5.7 Mb), as well as from other lower eukaryotes such as *Neurospora,* whose largest chromosomes are >10 Mb (Fan et al. 1989; Orbach et al. 1988). In *Drosophila,* a minichromosome of 1.3 Mb, containing centromeric and terminal regions of the *X* chromosome, has been resolved (Karpen and Spradling 1990), but to our knowledge there has been no investigation of chromosome *4* using PFGE.

We have obtained chromosome sized DNA from *D. melanogaster* cultured cells and embryos using simple isolation techniques. Using PFGE, Southern blotting, and probes for chromosome *4* specific gene sequences we have separated and identified chromosome *4*. This is the first description of *Drosophila* chromosome *4* using PFGE. A survey of different strains showed that the size of chromosome *4* varies with strain specificity from 4.5 to 5.2 Mb.

Materials and methods

Drosophila stocks. The *D. melanogaster* strains used in this study are described in Lindsley and Zimm (1992) and grown at 22° or 25° C on standard sucrose medium. Stocks were acquired from the Bowling Green, Umeå, or Indiana University Drosophila Stock centers except where noted.

Preparation of chromosome sized DNA. Drosophila Kc cells (Echalier 1976) were grown at room temperature in Shields and Sang M3 insect medium (Sigma) supplemented with 0.25% Difco Bactopeptone, 0.05% Difco Bacto TC-Yeastolate and 5% heat-inactivated calf serum. Chromosome sized genomic DNA was obtained from these cells by standard methods used for yeast and vertebrate cell lines (Schwartz and Cantor 1984).

Chromosome sized DNA was also obtained from embryos. Flies were permitted to lay eggs on grape agar plates for the times indicated (usually 6–18 h). Eggs were collected and washed with ice-cold 0.1% Triton-X100 solution and then dechorionated with bleach. After additional washing to remove the bleach, samples of 10–100 µl of eggs were gently disrupted using a plastic micro tissue homogenizer (no. 749520-0000, Kontes Scientific Glassware/Instrument, Vineland, N.J.) in 150 µl of Hanks' balanced salt solution (Gibco). This was quickly mixed with an equal volume of molten 1% Incert agarose (FMC) and poured into a plug forming mold (BioRad). After 10–20 min at 4° C the solidified plug was placed in 2–4 ml of ESP (0.1 M EDTA, 1% SDS, and 50 µg/ml Proteinase K) and incubated with gentle shaking at 45°–50° C for 48–72 h, after which plugs were stored at 4° C in this solution. Before loading into the pulsed field gel well, each plug was washed twice with the appropriate PFGE running buffer.

PFGE Procedures, Southern blots, and DNA probes. DNA was separated using a CHEF DR II apparatus (BioRad). A number of different conditions were used (see Results), however all gels were 0.7% agarose and run at 50 V at 14°–15° C. For 1 × TBE running buffer the run time was generally 7 to 8 days at the switch times indicated in Results. For a decreased run time, we used larger pore sized Chromosomal Grade Agarose (BioRad), 0.5 × TAE, and a shorter switch time. Bands were sized on autoradiographs by comparison with known chromosomes of *S. pombe* and the two largest chromosomes of *Saccharomyces cerevisiae.* Since high molecular weight bands near the origin can undergo mobility reversals, the identity of the *S. pombe* chromosome 1 at 5.7 Mb was always confirmed by probing with *S. pombe cdr1* DNA sequences, which are located between *cdc3* and *cdc16* on chromosome 1 (Feilotter et al. 1991).

Prior to vacuum transfer to GeneScreenPlus membrane (DuPont), DNA in the gels was depurinated (0.5 M HCl, 5–10 min) to facilitate transfer of large fragments. Probes were ^{32}P labeled by the oligo-labeling procedure of Feinberg and Vogelstein (1983, 1984) using DNA fragments isolated in low gel temperature agarose. Two probes from chromosome *4* were used: a 2.0 kb BglII fragment 5′ to the *cubitus interruptus* (*ci*) gene (Orenic et al. 1990; Locke and Tartof 1993) which will be referred to as the *ci* probe and a 3.6 kb EcoRI fragment containing sequences of a cDNA clone of the *zinc finger homolog-2* (*zfh-2*) gene (Lundell and Hirsh 1992; Fortini et al. 1991), which will be referred to as the *zfh-2* probe. Sequences for *ci* and *zfh-2* are located at 101EF and 102C, respectively, on chromosome *4*. For a control, non-chromosome *4* probe, an 8.5 kb SalI fragment containing the *yellow* gene and flanking sequences (Geyer and Corces 1987) was used and will be referred to as the *yellow* probe. *yellow* is located near the tip of the *X* chromosome, at 1B. The *rosy* gene sequences used were the 7.2 kb HindIII fragment from Carnegie 20 (Rubin and Spradling 1983). *rosy* is located at 87D on chromosome *3*.

These probe sequences are all single copy in the genome. When the same blot was hybridized sequentially to several probes, the previous probe was always removed first by treatment with 0.5 M NaOH at 42° C.

Results

Chromosome 4 from Drosophila Kc cell line DNA

The methodology for isolating chromosome sized DNA from yeast and mammalian cultured cells was used to prepare agarose-embedded DNA from a *D. melanogaster* Kc cell line. The separation of DNA from Kc cells, embedded at different concentrations, is shown in Fig. 1a. These PFGE conditions showed clear separation of the three *S. pombe* chromosomes (3.5, 4.6, and 5.7 Mb), which spans the estimated size of chromosome *4* (lane 5). No visible band corresponding to chromosome *4* was apparent in the Kc lanes of the ethidium bromide stained gel (lanes 3 and 4), although the small amount of sheared DNA present may have obscured a diffuse, faint band.

Probing a Southern blot of this gel with *ci* sequences (located on chromosome *4*) showed hybridization to a non-resolving zone of compression (zc) directly below the well and to a band at 5.2 Mb (Fig. 1b). Under certain PFGE conditions, a distinct zone of compression was not seen. When the zone of compression was absent, the *ci* probe hybridized to only the well and a 5.2 Mb band (data not shown). Reprobing the blot in Fig. 1 with the chromosome *4 zfh-2* sequences produced a pattern that could be superimposed and was indistinguishable in size and morphology from that of the *ci* probe (Fig. 1c). There were also several smaller faint bands evident, which were probably degradation artifacts. To show that hybridization to this 5.2 Mb band was specific for chromosome *4* probes, the blot was probed with *yellow* gene sequences from the *X* chromosome (Fig. 1d). Although hybridization signal was seen in the wells, zone of compression and diffused along the lane, no hybridization to the 5.2 Mb band was seen with the *yellow* probe. Preliminary experiments have indicated that a probe for the *rosy* locus on chromosome *3* also does not hybridize to the 5.2 kb band (data not shown). Because different amounts of DNA were loaded in adjacent lanes, the effect of concentration on mobility could be

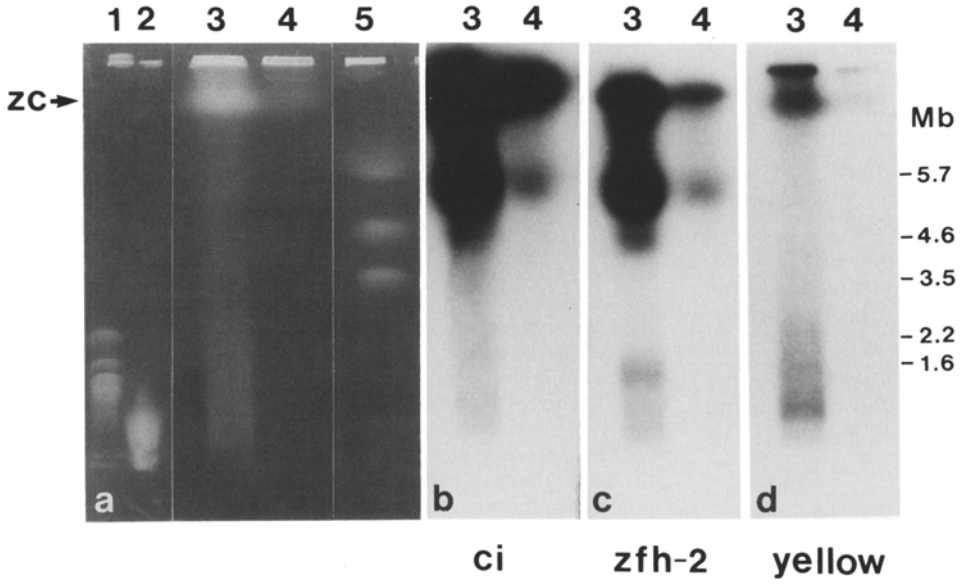

Fig. 1a–d. Separation of *Drosophila* chromosome *4* by pulsed field gel electrophoresis (PFGE) of Kc cell line DNA. DNA from agarose-embedded cells at two concentrations was used (*lane 3*, 6.2×10^6 cells/well; *lane 4*, 1.3×10^6 cells/well). Undigested DNA embedded in low gel temperature agarose was separated in a 0.7% agarose gel in 1 × TBE at 50 V for 180 h at 15° C with a linearly ramped switch time, which varied from 2500 to 4500 s. The DNA in gel **a** was transferred to a nylon membrane and consecutively probed with *ci* (**b**), *yellow* (**d**), and *zfh-2* (**c**). Bands were sized using *Schizosaccharomyces pombe* (*lane 5*) and *Saccharomyces cerevisiae* (*lane 1*) chromosomes as standards. Autoradiograms were compared by alignment of the lambda concatamer lane (no. *2*), which hybridized to radiolabeled lambda DNA present in the probe mixture. *zc* zone of compression. Exposure times: **b** 6 days, **c** 1 day, and **d** 14 days

examined. The mobility of the hybridization band was not significantly altered with these different concentrations of DNA from cultured Kc cells.

Results similar to those from Kc cells were seen with DNA preparations from Oregon R embryonic tissue. DNA from embryos of a *Drosophila* strain with a single P-element transposon insert, *ciD-placZ*, located on chromosome *4* (Eaton and Kornberg 1990), was separated and sequentially probed with *ci* and then with plasmid sequences that are present in the P-element insertion. Both probes produced a band of hybridization signal at about 5.2 Mb (data not shown), and these bands were superimposable, indicating that the plasmid sequences of the inserted P-element correspond to that of *ci*. Both have been localized to chromosome *4* (Eaton and Kornberg 1990; Locke and Tartof 1993).

The possibility of fragments even larger than the 5.2 Mb band was examined with PFGE conditions designed for this purpose (7000–10000 s; 1 × TBE; 168 h; Chromosomal Grade Agarose). In this gel the *S. pombe* chromosomes had migrated as an unseparated group of bands past the middle of the gel permitting larger fragments to resolve above (not shown). Owing to the lack of appropriate size markers we do not know the limit of resolution for this gel, but it is probably less than 10 Mb based on the conditions of Orbach et al. (1988). In this gel no chromosome *4*-specific band larger than the *S. pombe* region was seen.

The co-localization of the hybridization of three chromosome *4* sequences (*ci*, *zfh-2*, and plasmid sequences in *ciD-placZ*), but not the *X* chromosome *yellow* or chromosome *3 rosy* sequences, to the 5.2 Mb band is consistent with this band representing intact chromosome *4* separated from the other chromosomes on the gel.

Size variation of chromosome 4 between strains

Preliminary experiments with DNA of embryos suggested that the size of the chromosome *4* band varied slightly among different strains. To explore this size heterogeneity we examined DNA isolated from embryos of a variety of wild-type strains. Although the concentration and amount of shearing of the DNA varied with each preparation (Fig. 2a), a characteristic band showing hybridization with the *ci* and *zfh-2* probes but not *yellow* was seen in each lane (only *zfh-2* is shown; Fig. 2b). The size of the chromosome *4* band in these *D. melanogaster* strains varied from 4.5 to 5.2 Mb. Of the eight strains examined, the sizes appeared to fall into three groups: 4.5 Mb in strains Samarkand, $\pi 2$, and Urbana (lanes 2, 5, and 9 respectively), 4.8 Mb in strains Canton S and Harwich (lanes 7 and 8 respectively), and 5.2 Mb in strains Oregon R, Florida 9, and Crimea (lanes 1, 3, and 4 respectively). The observed differences in mobility appear to reflect genuine chromosome size heterogeneity for two reasons. First, the size differences do not correlate with the amount of DNA in the well or in the lanes suggesting this is not a factor in determining the mobility. Figure 1 shows that changes in DNA amount do not alter the mobility of the 5.2 Mb band. Second, these differences are reproducible. Different gels show repeatable differences between strains.

In addition to the various *D. melanogaster* strains,

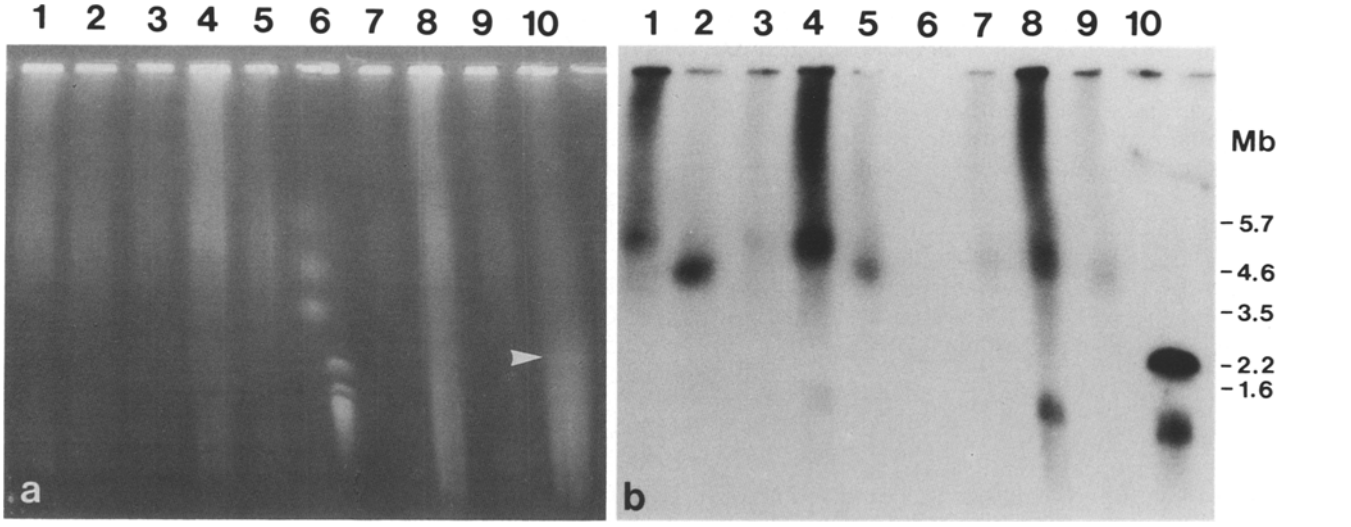

Fig. 2a, b. Variation in the size of chromosome *4* between different *Drosophila* strains and species. Undigested DNA of embryos embedded in low gel temperature agarose was separated in a 0.7% Chromosomal Grade Agarose gel in 0.5 × TAE at 50 V for 108 h at 14° C with a linearly ramped switch time, which varied from 1900 to 3400 s. The resulting blot was probed with *zfh-2* and the gel (**a**) and autoradiogram (**b**) are shown. Band sizes were determined by comparison with *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* chromosomes (*lane 6*, left and right side respectively). *Drosophila melanogaster* strains are as follows: *lane 1*, Oregon R; *lane 2*, Samarkand; *lane 3*, Florida 9; *lane 4*, Crimea; *lane 5*, π2; *lane 7*, Canton S; *lane 8*, Harwich; *lane 9*, Urbana. *Lane 10* contains DNA from a wild-type strain of *Drosophila simulans*. The *white arrowhead* in **a** indicates the position of the visible *D. simulans* ethidium bromide-stained band. Exposure time, **b** 10 h

a sample of a wild-type strain of the sibling species, *Drosophila simulans*, was examined using DNA prepared in the same manner (Fig. 2, lane 10). The fourth chromosomes in the two species are homologous and, consequently, the *D. simulans* homologs of the *zfh-2* and *ci* genes should be located on its chromosome *4*. The gene sequences between these species are highly conserved. We found the *zfh-2* and *ci* probes hybridized strongly to a band at 2.4 Mb in the *D. simulans* lane, approximately half the size of the *D. melanogaster* chromosome *4* band (only *zfh-2* is shown in Fig. 2). The additional weak hybridization signal below the 2.4 Mb band is due to a DNA migration artifact of compressed sheared DNA since it also hybridizes to the *yellow* probe (not shown). Unlike the *D. melanogaster* strains, the *D. simulans* lane lacks a smear upward from the chromosome *4* band. This indicates that, unlike the much larger *D. melanogaster* chromosome, a greater proportion of the *D. simulans* chromosomes may be entering the gel and that most of the chromosomes are entering the gel together. This may explain the distinct DNA band in the ethidium bromide stained gel that is visible in the *D. simulans* lane (white arrowhead). It corresponds exactly to the position of the chromosome *4* probe hybridization signal. We interpret this band as chromosome *4* DNA of *D. simulans*. We have not observed an ethidium bromide stained band of this clarity in the *D. melanogaster* chromosome *4* bands. The compression in the lower portion of this gel causes the largest two *S. cerevisiae* chromosomes to form sharp bands (Fig. 2, lane 6, right side) whereas the *S. pombe* chromosomes become more diffuse the larger they are (Fig. 2, lane 6, left side). The sharpness of the smaller sized *D. simulans* chromosome *4* band is presumably due to these specific PFGE conditions.

Discussion

Size and structure of chromosome 4

We have shown that two chromosome *4* sequences of *D. melanogaster* consistently hybridize to a band that is approximately 5.2 Mb, and that we feel corresponds to an intact chromosome *4*. This band is found in DNA isolated from both Kc cells and embryos. Thus this chromosome can be easily separated by the PFGE conditions previously determined for megabase sized *S. pombe* chromosomes. It appears that not all chromosome *4* DNA enters the gel from an agarose plug as there is considerable hybridization to DNA that either remains in the well or in the non-resolving zone of compression if one is present. In addition, there is often a smear between the well or zone of compression and the chromosome *4* band (Figs. 1, 2). This hybridization smear could be due to residual chromosomes that continue to escape from the well later in the run, after the initial flow of most chromosomes from the well has taken place.

The length of the euchromatic portion of chromosome *4* can be estimated. The 50 polytene bands on chromosome *4* account for 1% of the total number in the genome. The total length of the euchromatic banded region has been estimated to be about 110 Mb (Sorsa 1988), which makes the chromosome *4* portion about 1.1 Mb. An independent estimate of the euchromatic re-

gion, based on an average polytene band size of 21 kb (Spierer et al. 1983), gives a similar result of approximately 1.1 Mb for chromosome *4*. Our estimate of the total size of chromosome *4* compares well with, and refines, that obtained from ultraviolet absorbance measurements of metaphase chromosomes, which was 3.5% of the genome, or ~6 Mb (Rudkin as cited in Kavenoff and Zimm 1973). The difference between our total size estimate (4.5–5.2 Mb) and the length of euchromatic DNA sequences (1.1 Mb) probably represents heterochromatic sequences (3.4–4.1 Mb) that are underreplicated during polytene chromosome formation.

Strain-to-strain variation in size

A difference in chromosome *4* size was observed among the wild-type *D. melanogaster* strains. This variation is not due to mobility artifacts but represents genuine length differences among the chromosomes and presumably reflects differences in the amount of heterochromatic sequences. Were differences of this magnitude located in the euchromatic region the polytene banding pattern would surely be affected, however such strain specific differences are not seen. Simple satellite sequences have been localized to chromosome *4*. Specifically, the repeats $(AATAT)_n$ and $(AAGAG)_n$ hybridize to heterochromatic band regions 59 and 60, respectively (Lohe et al. 1993). The higher order size of these repeats has been estimated to be 2.7 and 0.17 Mb, respectively (Lohe et al. 1993). The production of whole chromosome length polymorphisms may be due to unequal crossing over, which is proposed to occur within such tandem arrays (Smith 1976). In fact, polymorphisms involving heterochromatic regions (which presumably contain tandem arrays of satellite sequences) are found in many species (reviewed by John and Miklos 1979). However, DNA sequence analysis of chromosome *4* euchromatic sequences suggested that this chromosome is unusual in that it lacks the extent of polymorphism found on other chromosomes (Berry et al. 1991). As chromosome *4* lacks normal recombination, the lack of euchromatic sequence polymorphism was attributed to a relatively recent "chromosome sweep", where the whole chromosome, acting as a single unit, spreads through a population. A similar lack of DNA sequence polymorphism was also observed among the fourth chromosomes from several *D. simulans* wild-type strains (Berry et al. 1991). Our observation of whole chromosome length polymorphisms may have arisen because we used wild-type strains isolated from diverse locations. This may have provided a more extensive sample in which polymorphisms could be found. Alternatively, these polymorphisms could have arisen since any chromosome sweep. The fact that the eight strains we examined fell into three size groups may indicate evolutionary relationships among chromosome *4* in these strains. If so, then the presence of P-elements in two strains ($\pi 2$ and Harwich) with different sized chromosomes suggests that the size change predates the arrival of P-elements as proposed by the recent invasion hypothesis for P-element distribution (Kidwell 1983). The heterochromatin polymorphisms we observed do not appear to have any phenotypic effect.

This strain-to-strain variation in chromosome size makes characterizing any previously described mutations that cytogenetically alter the polytene banding pattern of chromosome *4* equivocal. For example, we found that the mutation ey^D (*Tp(2;4)*ey^D, ey^D alp^{eD}), which has an insertion of about a dozen polytene bands, has a chromosome smaller than Oregon R wild-type upon PFGE analysis (data not shown). We suggest that the parental line for ey^D possessed a small chromosome *4* and that despite the insertion of chromosomal material, as seen by the presence of extra polytene chromosome bands, it is still smaller than Oregon R wild type. Assaying changes in chromosome *4* size due to mutations by PFGE would therefore require the parental strain for comparison if results are to be credible. This type of PFGE analysis will be useful for studying newly induced deletions, particularly large P-element mediated excisions.

Our estimate of the *D. simulans* chromosome *4* size is considerably smaller than that of *D. melanogaster* (2.4 vs 4.5–5.2 Mb). Comparison of the salivary gland polytene chromosomes between *D. melanogaster* and *D. simulans* shows a similar size and number of bands (reviewed in Lindsley and Zimm 1992) indicating that the euchromatic components of the chromosomes are similar. The difference in size we observed would suggest that there is a considerably smaller heterochromatic component to the *D. simulans* chromosome as compared with *D. melanogaster*. Such a difference may be relevant to the expression of genes on chromosome *4*. In a *D. melanogaster* genetic background in which one *D. simulans* chromosome *4* (*4-sim*) has been introgressed, the *D. simulans* chromosome *4* can substitute for almost all functions, indicating a similar genetic content to that of *D. melanogaster* chromosome *4* (Muller and Pontecorvo 1940). One exception to this complementation is a locus associated with male fertility: homozygous *4-sim* males are sterile (Muller and Pontecorvo 1942; Orr 1992). This locus has been mapped in *D. melanogaster* to the proximal region of the chromosome near the centromeric heterochromatin. Another exception is that the *4-sim* chromosome permits the expression of a recessive *cubitus interruptus* (*ci*) mutation when heterozygous (Uphoff 1949). Mutant expression of recessive *ci* mutations may be a chromosome pairing dependent phenomenon (Ephrussi and Sutton 1944; Locke and Tartof 1993). The disparity in size between the heterochromatic regions may affect the pairing of this locus, which is also located proximal on the chromosome, thereby influencing its expression.

The results show that PFGE could be used to determine the sizes of dot chromosomes in *Drosophila* species more accurately than classical cytogenetic methods. We have shown that the size varies between strains within one species and also varies between species. The size of the chromosome *4* homologs may also vary between strains in other species.

Use of PFGE in molecular mapping of Drosophila chromosome 4

The separation of *D. melanogaster* chromosome *4* by PFGE should advance the analysis of the chromosome as a whole. DNA preparations that show little shearing (e.g. Fig. 2, lane 2) will permit PFGE to be used as an analytical tool further to describe chromosome *4* length changes in any existing or future stock and to localize the site (or sites if they are dispersed along the chromosome) of these length variations. It should also be possible to use PFGE to isolate chromosome *4* DNA preparatively. This will facilitate the construction of chromosome specific clone libraries with which the molecular mapping of this chromosome can proceed. Currently there is a paucity of large cloned sequences from chromosome *4*. The smaller sized *D. simulans* chromosome *4* may offer additional material that can be used to facilitate and corroborate cloning and mapping efforts with *D. melanogaster* chromosome *4*.

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References

Berry AJ, Ajioka JW, Kreitman M (1991) Lack of polymorphism on the *Drosophila* fourth chromosome resulting from selection. Genetics 129:1111–1117

Eaton S, Kornberg TB (1990) Repression of *ci-D* in posterior compartments of *Drosophila* by *engrailed*. Genes Dev 4:1068–1077

Echalier G (1976) In vitro established lines of *Drosophila* cells and applications in physiological genetics. In: Kurstak E, Maramorosch K (eds) Invertebrate cell culture. Applications in medicine, biology and agriculture. Academic Press, New York, pp 131–150

Ephrussi B, Sutton E (1944) A reconsideration of the mechanism of position effect. Proc Natl Acad Sci USA 30:183–197

Fan JB, Chikashige Y, Smith CL, Niwa O, Yanagida M, Cantor CR (1989) Construction of a Not I restriction map of the fission yeast *Schizosaccharomyces pombe* genome. Nucleic Acids Res 17:2801–2818

Feilotter H, Nurse P, Young P (1991) Genetic and molecular analysis of *cdr1/nim1* in *Schizosaccharomyces pombe*. Genetics 127:309–318

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Feinberg AP, Vogelstein B (1984) Addendum: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267

Fortini ME, Lai Z, Rubin GM (1991) The *Drosophila zfh-1* and *zfh-2* genes encode novel proteins containing both zinc-finger and homeodomain motifs. Mech Dev 34:113–122

Geyer PK, Corces VG (1987) Separate regulatory elements are responsible for the complex pattern of tissue-specific and developmental transcription of the *yellow* locus in *Drosophila melanogaster*. Genes Dev 1:996–1004

Hochman B (1973) Analysis of a whole chromosome in *Drosophila*. Cold Spring Harbor Symp Quant Biol 38:581–589

John B, Miklos GLG (1979) Functional aspects of satellite DNA and heterochromatin. Int Rev Cytol 58:1–114

Karpen GH, Spradling AC (1990) Reduced DNA polytenization of a minichromosome region undergoing position-effect variegation in *Drosophila*. Cell 63:97–107

Kavenoff R, Zimm BH (1973) Chromosome-sized DNA molecules from *Drosophila*. Chromosoma 41:1–27

Kidwell MG (1983) Evolution of hybrid dysgenesis determinants in *Drosophila melanogaster*. Proc Natl Acad Sci USA 80:1655–1659

Lindsley DL, Zimm GG (1992) The genome of *Drosophila melanogaster*. Academic Press, San Diego

Locke J, Tartof KD (1993) Molecular cloning of *cubitus interruptus* (*ci*) mutations suggests an explanation for the ci-position effect. Mol Gen Genet (in press)

Lohe AR, Hilliker AJ, Roberts PA (1993) Mapping simple repeated DNA sequences in heterochromatin of *Drosophila melanogaster*. Genetics 134:1149–1174

Lundell MJ, Hirsh J (1992) The *zfh-2* gene product is a potential regulator of neuron-specific DOPA decarboxylase gene expression in *Drosophila*. Dev Biol 154:84–94

Miklos GLG, Yamamoto M-T, Davis J, Pirrotta V (1988) Microcloning reveals a high frequency of repetitive sequences characteristic of chromosome 4 and the β-heterochromatin of *Drosophila melanogaster*. Proc Natl Acad Sci USA 85:2051–2055

Muller HJ, Pontecorvo G (1940) Recombinants between *Drosophila* species the F1 hybrids of which are sterile. Nature 146:199–200

Muller HJ, Pontecorvo G (1942) Recessive genes causing interspecific sterility and other disharmonies between *Drosophila melanogaster* and *simulans*. Genetics 27:157

Orbach MJ, Vollrath D, Davis RW, Yanofski C (1988) An electrophoretic karyotype of *Neurospora crassa*. Mol Cell Biol 8:1469–1473

Orenic TV, Slusarski DC, Kroll KL, Holmgren RA (1990) Cloning and characterization of the segment polarity gene *cubitus interruptus Dominant* of *Drosophila*. Genes Dev 4:1053–1067

Orr HA (1992) Mapping and characterization of a 'speciation gene' in *Drosophila*. Genet Res 59:73–80

Roberts PA (1972) A possible case of position effect on DNA replication in *Drosophila melanogaster*. Genetics 72:607–614

Rubin GM, Spradling AC (1983) Vectors for P-element-mediated gene transfer in *Drosophila melanogaster*. Nucleic Acids Res 11:6341–6351

Schwartz DC, Cantor CR (1984) Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell 37:67–75

Smith JP (1976) Evolution of repeated DNA sequences by unequal crossover. Science 191:528–535

Sorsa V (1988) Chromosome maps of *Drosophila*, 2 volumes. CRC Press, Boca Raton, Florida

Spierer P, Spierer A, Bender W, Hogness D (1983) Molecular mapping of genetic and chromomeric units in *Drosophila melanogaster*. J Mol Biol 168:35–50

Uphoff DE (1949) The expression of alleles at the *cubitus interruptus* locus in hybrids between *Drosophila melanogaster* and *simulans*. Genetics 34:315–327