Chromosoma (1995) 103:539–544

CHROMOSOMA

A species specific satellite DNA family of *Drosophila subsilvestris* appearing predominantly in *B* chromosomes

Jutta Gutknecht, Diether Sperlich, Lutz Bachmann

Universität Tübingen, Biologische Fakultät, Lehrstuhl für Populationsgenetik,
Auf der Morgenstelle 28, D-72076 Tübingen, Germany

Received: 7 September 1994; in revised form: 1 November 1994 / Accepted: 2 November 1994

Abstract. This paper describes a species specific satellite DNA family (*pSsP216*) of *Drosophila subsilvestris*, a palearctic species of the *D. obscura* group. The *pSsP216* family consists of tandemly arranged 216 bp repetitive units that are predominantly localized on *B* chromosomes. These chromosomes appear in variable numbers in the karyotype of this species. Some *pSsP216* repeats can also be detected in the centromeric heterochromatin of the acrocentric *A* chromosomes. Two strains, one with and the other without *B* chromosomes, were investigated for sequence variability and for the location of this satellite DNA on the chromosomes. Among 16 clones of the 216 bp basic repeat unit an overall similarity of about 93% and no strain specific differences were found, indicating that the *B* chromosomes may have derived from the *A* chromosomes (probably the dots) by spontaneous amplification of the *pSsP216* satellite DNA family.

Introduction

The term "*B* chromosome" was introduced by Randolph (1928) in order to distinguish these small and morphologically specific chromosomes from the typically bigger "*A* chromosomes" in the chromosome complement of maize. *B* chromosomes are usually not necessary for cellular functions (Shaw and Hewitt 1990). During mitosis and meiosis these chromosomes are distributed to daughter cells by random segregation, and their number can, therefore, vary from individual to individual of the same species (Shaw and Hewitt 1990).

The presence of *B* chromosomes in chromosome sets is a rather widespread phenomenon, especially in plants (Puertas et al. 1987; Staub 1987). There are several examples known in insects (Shaw et al. 1985), but so far only one single case for the genus *Drosophila*, i.e. *Drosophila nasuta albomicans* (Ramachandra and Ranganath 1987; Hatsumi 1987).

The origin of *B* chromosomes is still mainly speculative, e.g. they may be derivatives of *X* chromosomes, or telocentric fragments arising through the mispairing of centromeres (Jones and Rees 1982). There is also very little known about possible effects of *B* chromosomes. Positive effects seem to be scarce; many deleterious effects on the fitness/survival of plants and insects have been observed (Jones and Rees 1969; Hewitt 1979; Beukeboom and Werren 1993).

By means of specific staining techniques it is possible to demonstrate that *B* chromosome are basically heterochromatic and that they might consequently consist to a large degree of highly repetitive satellite DNA.

Here we describe the identification of supernumerary *B* chromosomes in *D. subsilvestris*, a palearctic species of the *D. obscura* group. Its haploid karyotype has been described as consisting of only two metacentric, one submetacentric, two acrocentric and one dot chromosomes (Lakovaara and Saura 1982). In the present study we will show that supernumerary *B* chromosomes can be found in strains directly derived from nature. Since this is a unique situation in the *D. obscura* species group a molecular analysis was performed in addition.

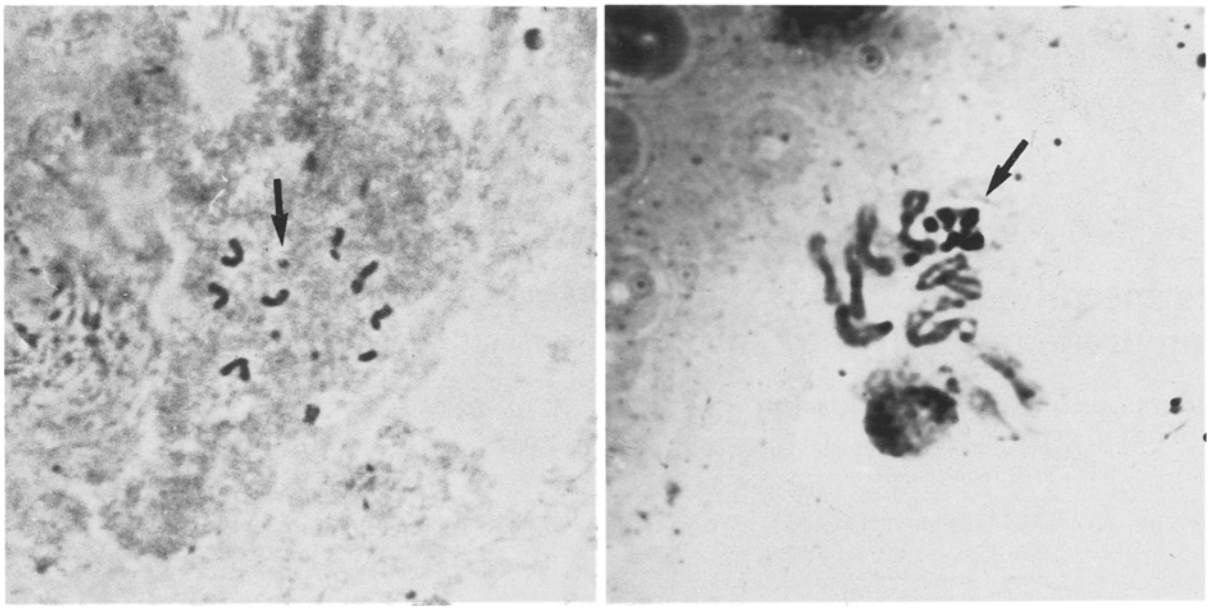
Materials and methods

Drosophila strains. Two different strains of *D. subsilvestris* were used. Both strains were established as mass cultures from wild flies caught in wooded areas near Tübingen, Germany. Strain A was kept in the laboratory from 1978 and died out in 1992; strain B was established in autumn 1992. The strains have been kept in the laboratory in continuous light and at a constant temperature of 18° C.

DNA isolation and cloning. Genomic DNA of *Drosophila* was extracted as described by Preiss et al. (1988). highly repetitive DNA was isolated from satellite DNA bands visible after 5% polyacrylamide gel electrophoresis of PvuII digested genomic DNA. The DNA fragments were eluted from the gel, purified and ligated into the plasmid pUC19 (King and Blakesley 1986), and transformed

Edited by: E.R. Schmidt

Correspondence to: L. Bachmann



A B

Fig. 1A, B. Mitotic chromosome sets of *Drosophila subsilvestris* from strain A (**A**) and at a higher magnification from strain B. (**B**) The preparations were made shortly after establishing the strains in 1978 (**A**) and 1992 (**B**). *Arrows* indicate the supernumerary *B* chromosomes. Magn. 1000 ×

into *Escherichia coli* JM103 cells. Recombinant clones were selected as white colonies on ampicillin plates containing X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) and IPTG (isopropyl-β-D-thiogalactopyranoside).

DNA sequencing. The nucleotide sequences were determined by the dideoxy chain termination method (Sanger et al. 1977) using the USB sequenase kit (No. 70770) for ^{35}S radioactive sequencing and the Autoread kit (Pharmacia No. 27-1690-04) for automatic sequencing on an A.L.F. (Pharmacia). DNA for the sequencing reactions was prepared according to Lee and Rasheed (1990).

Filter hybridization. Fragments of digested DNA were separated by agarose gel electrophoresis and transferred to Hybond N membranes (Amersham) by the method of Southern (1975). Labeling of probe DNA with Digoxigenin and signal detection were carried out according to the instructions accompanying the "DIG DNA labeling and detection kit-nonradioactive" (Boehringer No. 109 36 57) and according to Kreike et al. (1990).

Preparation of mitotic chromosomes. Neural ganglia of third instar larvae were dissected in *Drosophila* Ringer (5.55 g NaCl, 0.22 g KCl, 0.44 g $CaCl_2$, 1000 ml distilled water) and then treated for 20 min in a hypotonic solution of 1% sodium citrate. After 10 min fixation in a cold 3:1 ethanol:acetic acid mixture the ganglia were resuspended in a drop of 45% acetic acid and spread onto a clean slide prewarmed to 42° C. After drying, the chromosomes were dehydrated in ethanol (70%, 90%, 100%) and then air dried.

In situ hybridization of chromosome preparations. Fifteen microliters of a 50% formamide, 5% dextran sulfate, 4×SSC and DIG-labeled DNA (1 ng/μl) mixture were placed on a slide, covered with a coverlip and sealed with rubber cement. (1×SSC is 0.15 M NaCl, 0.015 M sodium citrate). The probe and the chromosomal DNA were denatured simultaneously at 80° C for 10 min. In situ hybridization was performed overnight in a humid box at 37° C. Unspecifically bound DNA was removed by washing the slides in 2×SSC for 5 min at room temperature, twice for 10 min at hybridization

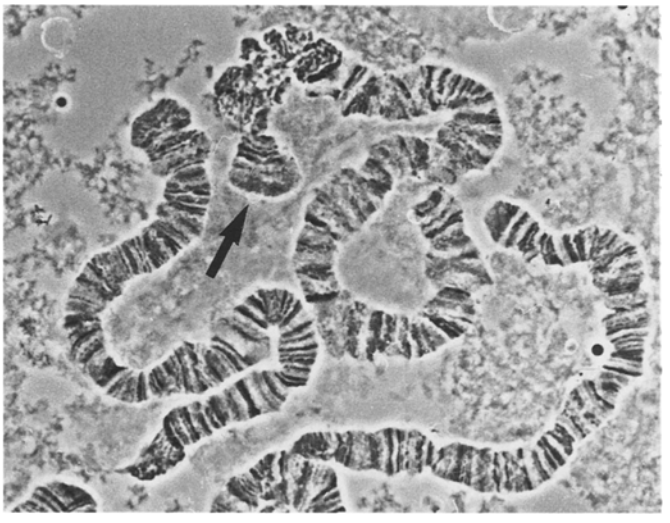


Fig. 2. Part of a set of polytenic chromosomes of *D. subsilvestris* from larval salivary gland tissue. The degree of polytenization of the dot chromosome (indicated by an *arrow*) is the same as that of the other chromosomes. Magn. 500 ×

temperature and once more for 5 min at room temperature in 2×SSC. Subsequently, the slides were washed briefly in 100 mM Tris-HCl, 150 mM NaCl, 0.3% Tween 20, pH 7.5 and preincubated in 100 mM Tris-HCl, 150 mM NaCl, 0.3% Tween 20, pH 7.5 containing 0.5% blocking reagent (Boehringer) at 37° C for 30 min. The anti-DIG-peroxidase conjugate (in 100 mM Tris-HCl, 150 mM NaCl, pH 7.5 containing 0.5% blocking reagent) was placed on the slide with the chromosomes and incubated for 45 min at 37° C followed by three washes in 100 mM Tris-HCl, 150 mM NaCl, pH 7.5. The detection was done in 50 mM Tris-HCl, 0.05% diaminobenzidine, 0.01% H_2O_2 pH 7.5 for 10 min in the dark. The slides

```
            1          11         21         31         41         51
pSsP216/1   CAGCTGTATT CAGCGGACCA AACCAGGAGT CAACCCCGAA GCAACGGTCA CGGACAGAGA
pSsP216/2   ---------- ---------- ---------- ---------- --------T- ----------
pSsP216/3   ---------- ---------- ---------- ---------C ---------- ----------
pSsP216/4   ---------- ---------- ---------- ---------- ----T----- ----------
pSsP216/5   ---------- ---------- ---------- ---------- ---------- ------T---
pSsP216/6   ---------- ---------- ---------- ---------- ---------- ----------
pSsP216/7   ---------- ---------- ---------- ---------- ---------- ----------
pSsP216/8   ---------- ---------- ---------- ---------C ---------- ----------
pSsP216/9   ---------- ---------- ---------- ---------- ----T----- ----------
pSsP216/10  ---------- ---------- ---------- ---------- ---------- ----------
pSsP216/11  ---------- ---------- ---------- ---------- ----T----- ----------
pSsP216/12  ---------- ---------- ---------- -------A-- ----T----- ----------
pSsP216/13  ---------- ---------- ---------- -------A-- ---------- ------T---
pSsP216/14  ---------- ---------- ---------- ---------- ---------- ----------

            61         71         81         91         101        111
pSsP216/1   CTTTGGGAGC GGCAACCACA CCCAGTGTGC CAAACCAGGA GCCTCATGCT CAGACAACTT
pSsP216/2   ------C--- ---------- ---------- ---------- ---------C ----------
pSsP216/3   -----TC--- ---T------ ---------- ---------T ---------C ----------
pSsP216/4   -----TC--- ---------- ---------- ---------T ---------C ----------
pSsP216/5   ------C--- ---------- -T---G---- ---------- ---------C ----------
pSsP216/6   ------C--- ---------- ---------- ---------- ---------C ----------
pSsP216/7   ------C--- ---------- ---------- ---------- ---------C ----------
pSsP216/8   -----TC--- ---------- ---------- ---------T ---------C ----------
pSsP216/9   T----TC--- ---------- ---------- ---------- ---------C ----------
pSsP216/10  ------C--- ---------- ---------- ---------- ---------C ----------
pSsP216/11  T----TC--- ---------- ---------- ---------- ---------C ----------
pSsP216/12  -----TC--- ---------- ---------- ---------T ---------C ----------
pSsP216/13  ------C--- ---------- -T---G---- ---------- ---------C ----------
pSsP216/14  ------C--- ---------- ---------- ---------- ---------C ----------

            121        131        141        151        161        171
pSsP216/1   CAC*GGGCAG GAACTTTGGC AAAGACGGCC AACAACAGCG CGTCAAACCA GGAGTCACCT
pSsP216/2   ---T------ --------TT G----GA--- ---------- ---------- ----------
pSsP216/3   ---*------ ---A------ -G-------- --A---G--- ---------- ----------
pSsP216/4   ---*------ ---------- -G-------- ------G--- ---------- ----------
pSsP216/5   ---*------ --------TT -C-------- ---------- ---------- ----------
pSsP216/6   ---*------ ---------- -G-------- --------T- ---------- ----------
pSsP216/7   ---*------ ---------- -G-------- --------T- ---------- ----------
pSsP216/8   ---*------ ---T------ -G-------- --T------- ---------- A---------
pSsP216/9   ---*------ ---------- -G-------- ---------- ---------- ----------
pSsP216/10  ---*------ ---------- -G-------- --------T- -A-------- ----------
pSsP216/11  ---*------ -------A-- -G-------- ---------- ---------- ----------
pSsP216/12  ---*------ ---------- -G-------- ---------- ---------- ----------
pSsP216/13  ---*------ ---------- -G-------- ---------- ---------- ----------
pSsP216/14  ---*--C--- ---------- -G-------- ---------- ---------- -----AT---

            181        191        201        211
pSsP216/1   GCAACACCAC GATCCCAGGC AAAGATTTTG GCAAAGG
pSsP216/2   ---------- ---------- -----C---- -------
pSsP216/3   -------A-- ---------- -----C---- -------
pSsP216/4   ---------- ---------- -----C---- -------
pSsP216/5   ---------- ---------- -----C---- -------
pSsP216/6   ---------- -----T---- -----C---- ---G---
pSsP216/7   -------T-- ---------- -----C---- -------
pSsP216/8   ---------- ---------- -----C---- -------
pSsP216/9   ---------- ---------- -----C---- -------
pSsP216/10  ---------- ---------- -----C---- -------
pSsP216/11  ---------- ---------- -----C---- -------
pSsP216/12  ---------- ---A------ -----C---- -------
pSsP216/13  ---------- ---------- -----C---- -------
pSsP216/14  ---------- -------*--- -----C---- -------
```

Fig. 3. Nucleotide sequences of *pSsP216/1–14* from *D. subsilvestris* aligned with *pSsP216/1*. *Asterisks* indicate gaps introduced to increase sequence similarity

were air dried and analyzed microscopically with reflection contrast (Landegent et al. 1985).

Preparation of salivary gland chromosomes. The usual squashing method for polytene chromosomes of *Drosophila* was used (Ashburner 1989). The chromosomes were stained in a 60% acetic orcein solution.

Results

The configurations of the mitotic chromosome sets of *D. subsilvestris* (strains A and B) are shown in Fig. 1. They consist of two pairs of acrocentric chromosomes, two pairs of metacentric chromosomes (one of them is the *X* chromosome) and one pair of submetacentric, *Y*-shaped chromosomes. In addition a variable number (one to five) of dot-like chromosomes was observed in most of the chromosome sets studied. With respect to these dot-like chromosomes we found differences between strains A and B. Up to five dot-like chromosomes appeared in chromosome preparations prepared shortly after strain A had been established from wild flies; only one or none at all were found after several years of laboratory maintenance. In the newly established strain B, however, a variable number of dot-like chromosomes was again present in mitotic metaphases. In preparations of polytene chromosomes (Fig. 2) from salivary glands, however, only

one small banded dot chromosome could be seen irrespective of the number of dot-like chromosomes appearing in the metaphases. From this observation it was concluded that the supernumerary dot-like chromosomes of *D. subsilvestris* must be *B* chromosomes. *B* chromosomes are absent in almost all chromosome sets of *Drosophila*. So far, they have only been observed in *D. nasuta albomicans* (Ramachandra and Ranganath 1987; Hatsumi 1987). Since only one polytene dot chromosome with a normal degree of polytenization occurred in each salivary gland nucleus it was further assumed that the supernumerary *B* chromosomes are heterochromatic and contain repetitive sequences that are underreplicated and hence not visible in polytenic nuclei (Zacharias 1993).

To obtain more information, a search for repetitive sequences in the genome of *D. subsilvestris* was performed. Genomic DNA of the two strains (A, B) of *D. subsilvestris* was digested with a number of different restriction endonucleases and subsequently electrophoresed on a 5% polyacrylamide gel; only PvuII and HaeIII digested DNA, respectively, gave the ladder-like pattern that is typical of tandemly arranged satellite DNA. The DNA of the smallest PvuII restriction satellite fragment (about 200 bp) was subsequently eluted from the gel and ligated into the plasmid vector pUC19. After transformation seven positive clones were identified from each strain and sequenced: *pSsP216/1–7* from population A and *pSsP216/8–14* from population B. All sequences had a length of 216 bp, apart from clones *pSsP216/2* and *pSsP216/14*, which were 217 and 215 bp in length, respectively. The sequences showed an average homology of 96.35% (93.52%–99.54%) and were thus considered as members of the *pSsP216* satellite DNA family (Fig. 3). The *pSsP216* repeats are slightly GC rich (55%) and exhibit a remarkably asymmetric base composition, especially with respect to A and T nucleotides (A, 31.5%; C, 30.5%; G, 25.5%; T, 12.5%). As expected from the electrophoresis data all PvuII repeats have an internal HaeIII restriction site (Fig. 3, position 147–150). The analyzed *pSsP216* sequences from the two different lines of *D subsilvestris* exhibited no strain specific variation. An EMBL data bank search revealed no significant similarity of *pSsP216* sequences to any other known nucleotide sequence.

In order to test whether the sequences of the *psSp2167* family are also present in the genomes of other species of the *D. obscura* group, genomic DNA of *D. madeirensis, D. obscura, D. ambigua, D. tristis, D. subobscura, D. pseudoobscura, D. bifasciata* and *D. subsilvestris* (strains A and B) was digested with the restriction endonuclease PvuII, electrophoresed on a 1% agarose gel and blotted. The hybridization with labeled *pSsP216/3* DNA revealed that the ladder-like pattern of typical satellite DNA occurred only with DNA from *D. subsilvestris*. A significant hybridization signal could be observed with DNA fragments of high molecular weight from *D. ambigua*. Interestingly, the hybridization signal with DNA from strain A was much weaker (i.e. almost invisible) compared with that with DNA from strain B, although almost the same amount of genomic DNA was used. This was completely unexpected as almost no difference in the intensity of the ~200 bp PvuII restriction satellite band on 5% polyacrylamide gels was observed between strain A and B. The solution to this discrepancy was that genomic DNA of strain A was used that came from two different preparations: the DNA of strain A used for the identification of the *psSp216* satellite DNA by polyacrylamide gel electrophoresis was prepared in 1986 when strain A had been kept for 8 years in the laboratory and the DNA of stock A used for the Southern blot analysis was prepared in 1992 after 14 years of cultivation of strain A. Therefore, most of the *pSsP216* sequences were probably eliminted from the genome of strain A during this period of cultivation. This was also the case for the *B* chromosomes.

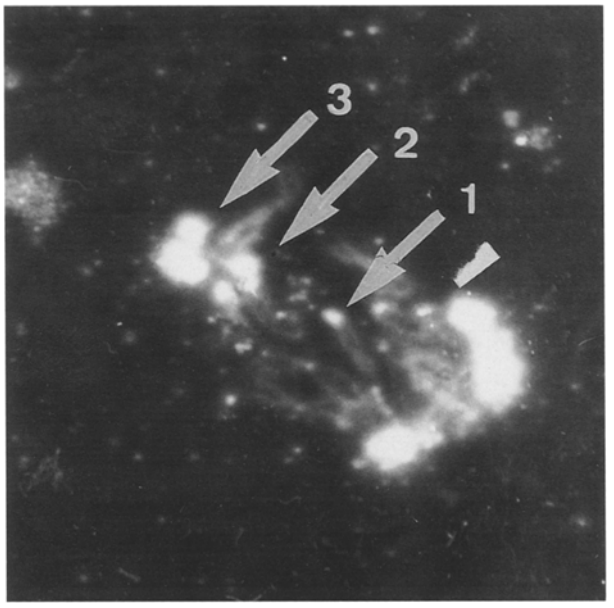


Fig. 4. In situ hybridization of clone *pSsP216/3* to mitotic chromosomes of *D. subsilvestris* (strain B) larvae. Hybridization signals are visible on the centromeres of the acrocentric chromosomes (*1*), on the dot chromosomes (*2*) and on the *B* chromosomes (*3*). Magn. 1000 ×

A rough quantification of the contribution of *pSsP216* sequences to the genome of *D. subsilvestris* was performed by means of dot-blot hybridizations using cloned *pSsp216/3* DNA and genomic DNA from strain B. The resulting estimate of 0.5%–1% as an average proportion of *pSsP216* sequences in the genome (data not shown) is of course only valid for the specific strain B in summer 1993, when the genomic DNA was prepared.

For chromosomal localization of the *pSsP216* sequences in situ hybridizations to mitotic chromosomes prepared from larvae of strain B were carried out, using clone *pSsP216/3* as a probe. Weak hybridization signals were visible with reflection contrast microscopy on the centromeres of the acrocentric chromosomes and strong ones on the dot chromosomes. In addition, extremely strong signals appeared that were so intense that it was difficult to associate them with any underlying chromo-

somal structure. Therefore, chromosomes of the same slides were stained with Giemsa and inspected in the normal light microscope. A comparison showed that all structures that produced intense hybridization signals were dot-like *B* chromosomes (Fig. 4).

Discussion

In this study a species-specifically amplified satellite DNA family *pSsP216* could be detected. It represents a major component of additional *B* chromosomes of the chromosome complement of *D. subsilvestris*. Copies of the same family were also found in the centromeric heterochromatin of the two acrocentric chromosomes and on the dot chromosomes. The *pSsP216* satellite DNA family shows all the characteristics of a typical satellite DNA: a repetitive unit of discrete length (216 bp), high intraspecific sequence homogeneiy (96.35%) and a clear tandemly repeated arrangement in the genome.

B chromosomes and dot chromosomes cannot be distinguished in the microscope without specific staining techniques. One pair of dot chromosomes appears in all karyotypes of the species of the *D. obscura* group. C-banding techniques showed that these dots are rather heterochromatic in all the species of the *D. obscura* group (Raab 1988). The dot chromosomes of *D. subsilvestris* stained with the fluorescent dyes 4,6-diamidino-2-phenylindole (DAPI)/Chromomycin A_3 (CMA) exhibit GC-rich DNA in the centromeric and AT-rich DNA in the distal regions, a pattern that was not observed in dot chromosomes of other *D. obscura* group species (Raab 1988) at all. Clusters of the slightly GC-rich *pSsP216* sequences (55%) are probably limited to the centromeric region of the dot chromosomes. However, if additional *B* chromosomes are present, extremely intense hybridization signals occur. We therefore believe that the *B* chromosomes contain either very big clusters of amplified *pSsP216* sequences or that they are entirely composed of them.

Based on these results we suggest that the *B* chromosomes may have originally arisen from the dots. However, dot chromosomes behave like the other normal *A* chromosomes during meiosis while the *B* chromosomes seem to segregate randomly, leading to different *B* chromosome numbers in different individuals (varying at least from zero to four, according to our observations). The mechanism responsible for the origin of *B* chromosomes in *D. subsilvestris* might be inferred from the experiments of Haaf et al. (1992). After transformation of in vitro cultured African green monkey cells with human alpha satellite sequences they found that clusters of human alpha satellite sequences of approximately 15 kb were amplified in some instances up to 200 kb. Furthermore, so-called double minutes appeared and persisted after transformation over several cell generations. The double minutes exhibited "normal" replication and segregation behavior. In situ data showed that these double minutes consisted of human alpha satellite DNA. Although speculative, it appears possible to explain the origin of *B* chromosomes of *D. subsilvestris* in a similar way by extrachromosomal amplification of *pSsP216* sequences. If so, we have to conclude that the nucleotide sequence of *pSsP216* repeats and their tandemly repeated organization carry information necessary for establishing a chromosome-like structure in combination with specific proteins – at least a structure that fulfills the requirements of a centromere/kinetochore. The presence of *pSsP216* sequences in the centromeric regions of the two acrocentric chromosomes and the normal dot chromosomes may be taken as support for the assumption that clusters of *pSsP216* sequences might possess some centromere-like properties. The problem with this hypothesis is that a structure that only contains amplified *pSsP216* repeats lacks telomeres and should, therefore, degenerate. If we assume continuous elongation of the *pSsP216* arrays by an amplification mechanism on the one hand and continuous reduction in length due to the lack of telomeres on the other an equlibrium situation could be imagined. Therefore, it may be possible to achieve an almost constant size of *B* chromosomes.

In comparison with other satellite DNAs described so far for *D. obscura* group species, the *pSsp216* repeats reveal no peculiarities that would classify them as an extraordinary satellite DNA. Certainly, the GC content of roughly 55% is slightly though not remarkably higher than that of other satellite DNA families of the centromeric heterochromatin, i.e. 27% in the KM190 family of *D. kitumensis* and *D. microlabis* (Bachmann et al. 1992) 30% in the *pGH290* family of *D. guanche* (Bachmann et al. 1989), and 40%–45% in the *ATOC180* family of *D. ambigua, D. tristis* and *D. obscura* (Bachmann and Sperlich 1993). On the other hand, the observed average variability of 3.65% between repeats is smaller than in any of the other satellite DNAs of the other species of the group, i.e. 5%–6.7% in the *KM190* family, 11.3% in the *pGH290* family, and 7%–15% in the *ATOC180* family. Computer simulations show that such a low value of variability between repeats may be the result of a rather high recombination rate (Stephan and Cho 1994). Furthermore, the model of Stephan and Cho (1994) predicts that increasing rates of unequal exchanges lead to lower copy numbers per cluster. Indeed, the copy number of *pSsP216* repeats seems to be low compared with those of the *KM190, ATOC180* and especially the *pGH290* repeats if we exclude the *B* chromosomes. However, the data are difficult to compare for two reasons: (i) in addition to general methodological problems in the estimation of copy numbers per genome, the obtained values for the *pSsP216* repeats are only of limited value owing to the variable number of *B* chromosomes and (ii) the *KM190, ATOC180* and *pGH290* satellite DNAs were detected in the centromeric heterochromatin of all chromosomes (except the *Y*) of the respective species. Therefore, it may be possible that the copy numbers per genome are higher but that the actual sizes of the tandemly arranged arrays are smaller. Copies of the *pSsP216* satellite DNA family derived from *B* chromosomes should in any case be homogeneous if the origin of *B* chromosomes is spontaneous extrachromosomal amplification of a chromosomal template.

Acknowledgements. We thank Michael Raab-Vetter for technical instructions for the in situ hybridization experiments. We also thank Wolfgang Stephan for valuable suggestions for improving the manuscript.

References

Asburner M (1989) *Drosophila*: A laboratory manual. Protocol 17 Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Bachmann L, Sperlich D (1993) Gradual evolution of a specific satellite DNA family in *Drosophila ambigua, D. tristis* and *D. obscura*. Mol Biol Evol 10:647–659

Bachmann L, Raab M, Sperlich D (1989) Satellite DNA and speciation: a species specific satellite DNA of *Drosophila guanche*. Z Zool Syst Evol–Forsch 27:84–93

Bachmann L, Müller E, Cariou ML, Sperlich D (1992) Cloning and characterization of KM190, a specific satellite DNA family of *Drosophila kitumensis* and *D. microlabis*. Gene 120: 267–269

Beukeboom LW, Werren JH (1993) Deletion analysis of the selfish B chromosome, paternal sex ratio (PSR), in the parasitic wasp *Nasonia vitripennis*. Genetics 133:637–648

Haaf T, Warburton PE, Willard HF (1992) Integration of human alpha-satellite DNA into simian chromosomes: Centromere protein binding and disruption of normal chromosome segregation. Cell 70:681–696

Hatsumi M (1987) Karyotype polymorphism in *Drosophila albomicans*. Genome 29:395–400

Hewitt GM (1979) Orthoptera: Grasshoppers and crickets. Animal Cytogenetics 3: Insecta 1. Bornträger, Berlin

Jones RN, Rees H (1969) An anomalous variation due to B chromosomes in rye. Heredity 24:265–271

Jones RN, Rees H (1982) B chromosomes. Academic Press, New York

King PV, Blakesley RW (1986) Optimizing DNA ligation for transformations. Focus 8:1–3

Kreike CM, de Koning JRA, Krens FA (1990) Non-radioactive detection of single-copy DNA-DNA hybrids. Plant Mol Biol Rep 8:172–179

Lakovaara S, Saura A (1982) Evolution and speciation in the *Drosophila obscura* group. In: Ashburner M, Carson HL, Thompson JN Jr (eds) Genetics and biology of *Drosophila*, vol 3b. Academic Press, New York London, pp 1–59

Landegent JE, Jansen in de Wal N, van Ommen GJB, Baas F, de Vijlder JJM, van Duijn P, van der Ploeg M (1985) Chromosomal localization of a unique gene by non-autoradiographic in situ hybridization. Nature 317:175–177

Lee S, Rasheed S (1990) Mini-screening of recombinant bacteria. Bio Techniques 9:676–679

Preiss A, Hartley DA, Artavanis-Tsakonas S (1988) Molecular genetics of enhancer of split, a gene required for embryonic neural development in *Drosophila*. EMBO J 12:3917–3927

Puertas MJ, Ramirez A, Baeza F (1987) The transmission of B chromosomes in *Secale cereale* and *Secale vavilovii* populations. II. Dynamics of populations. Heredity 58:81–86

Raab M (1988) Untersuchung des Heterochromatins durch Bänderungstechniken und cytologische Lokalisierung von hochrepetitiven DNA-Sequenzen in Arten der *Drosophila obscura* Gruppe. Diploma thesis, University of Tübingen

Ramachandra NB, Ranganath HA (1987) Characterization of heterochromatin in the B chromosomes of *D. nasuta albomicans*. Chromosoma 95:223–226

Randolph LF (1928) Types of supernumerary chromosomes in maize. Anat Rec 41:102

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Shaw MW, Hewitt GM (1990) B chromosomes, selfish DNA and theoretical models: where next? In: Futuyama D, Antonovics J (eds) Oxford surveys in evolutionary biology, vol 7. Oxford University Press, Oxford, pp 197–223

Shaw MW, Hewitt GM, Anderson DA (1985) Polymorphism in the rates of meiotic drive acting on the B chromosome of *Myrmeleotettix maculatus*. Heredity 55:61–68

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Staub RW (1987) Leaf striping correlated with the presence of B chromosomes in maize. J Hered 78:71–74

Stephan W, Cho S (1994) Possible role of natural selection in the formation of tandem-repetitive noncoding DNA. Genetics 136:333–341

Zacharais H (1993) Larger nuclei in the larval brain of *Drosophila nasutoides* often shows underreplication, whereas metaphases provide a reliable DNA standard. Genome 36:294–301