Sequence Instability and Functional Inactivation of Murine Y Chromosomes Can Occur on a Specific Genetic Background

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When the Y chromosome from Mus. poschiavinus (Y\textsuperscript{Pos}) is backcrossed onto the C57BL/6J laboratory strain, testicular dysfunction occurs at high frequencies. When five different multicopy probes from the recombinationally suppressed region of the Y chromosome were used, genomic DNAs from sibling female progeny of C57BL/6J Y\textsuperscript{Pos} males were found to contain Y\textsuperscript{Pos}-specific sequences ranging from trace levels to levels consistent with an intact Y chromosome. Females with a high copy number of Y\textsuperscript{Pos}-specific sequences had a karyotype of XY\textsuperscript{Pos} and were sterile. Females with trace levels of these sequences were XX and fertile. Repeated sequences in the testis-determining-region (Sxr) of inactive Y\textsuperscript{Pos} chromosomes were unstable relative to sequences in non-Sxr regions. In contrast, the Y\textsuperscript{Pos} chromosome was stable and functioned normally in other inbred laboratory strains such as 129/Sv. The frequency and extent of Y\textsuperscript{Pos} chromosome instability increased with successive backcrosses from stable (129/Sv) to unstable (C57BL/6J) genetic backgrounds. Traces of Y\textsuperscript{Pos}-specific sequences were first detected in N2 female offspring of F1 males. Therefore, sequences were deleted from Y\textsuperscript{Pos} chromosomes in the F1 male germ line and were transmitted to N2 females; inactive Y\textsuperscript{Pos} chromosomes (XY\textsuperscript{Pos} females) were first detected in the N3 generation. The mouse line being derived by backcrossing the Y\textsuperscript{Pos} chromosome onto C57BL/6J inbred strains ended in the N7 generation, since all XY\textsuperscript{Pos} offspring were sterile. Even stable repeated sequences from the non-Sxr regions of their inactive Y\textsuperscript{Pos} chromosomes were precisely rearranged in these N7 offspring at high frequencies. These data are consistent with hybrid dysgenesis in mammals.

Introduction

Haldane first observed that, when hybrids of evolutionarily diverged species or races are either sterile or inviable, they belong to the heterogametic (XY or ZW) sex, rather than to the homogametic (XX or ZZ) sex (Haldane 1922). It was subsequently shown that both genic and chromosomal incompatibilities were responsible for inviability and infertility in hybrids of Drosophila species (Sturtevant 1939; Dobzhansky 1951).

Furthermore, it is recognized that the heterogametic sex- and genetic-background-dependent induction of specific genotypic and phenotypic traits constitutes hybrid dysgenesis in Drosophila (Hiraizumi 1971; Kidwell et al. 1977; Bregliano et al. 1980; Bingham et al. 1982). A major genotypic trait characterizing hybrid dysgenesis is the

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generalized induction of recombination (Hiraizumi 1971). This loss of control results from the availability of breakpoints at sites of excision of transposons but also includes suppression of recombination, transmission-ratio distortion, translocations, aneuploidies, and high frequencies of mutations (Hiraizumi 1971; Kidwell et al. 1977; Hinton 1979; Bregliano et al. 1980; Bingham et al. 1982; Yannopoulos et al. 1987). Mutations of gonadal functions are also detected frequently in hybrid genomes undergoing random recombination because of (1) their nonlethal effects, (2) the large numbers of encoding target loci, and (3) the simplicity of monitoring mutant phenotypes (e.g., by sex ratios, external genitalia, fertility, and karyotypic analysis) (Kidwell et al. 1977; Bingham et al. 1982; Yannopoulos et al. 1987).

Similar genotypic and phenotypic traits have been reported in hybrids or strains derived from hybrids of several murine species. Chromosomal aneuploidy and embryonal inviability (Gropp et al. 1972; Cattanach and Moseley 1973; Capanna 1982), sterility (Gropp et al. 1972; Cattanach and Moseley 1973; Forejt and Iványi 1975; Capanna 1982; Handel 1987), and sex reversal (Eicher and Washburn 1986) occur at high frequencies. Testicular dysfunction in these hybrids or strains, however, has not been molecularly correlated to mutations resulting from induction of sequence instability. The presence of major testicular determinants on the murine Y chromosome has now been established by the canonical loss-of-function/gain-of-function analyses. This locus is referred to as testis-determining Y-linked, or Tdy (Eicher and Washburn 1986), and is contained within the Sxr region of the murine Y chromosome, which on translocation causes XX maleness (Cattanach et al. 1982; Evans and Burtenshaw 1982; McLaren and Monk 1982; Singh and Jones 1982; McLaren et al. 1988 and Roberts et al. 1988). The necessity for sequestering the Y-linked copies of the testis-determining locus from the female genome is illustrated by the dominant maleness of the sterile translocation mutant XXsor (Cattanach et al. 1982; McLaren and Monk 1982; Singh and Jones 1982). This sequestration is thought to be effected by the male-limited haploidy and by the suppression of interchromosomal X-Y recombination over the predominant length of the Y chromosome (Tres 1977; Chandley et al. 1984).

High frequencies of sex reversal (XY females) result when the Y chromosome from the feral species Mus poschiavinus (Ypos chromosome) is backcrossed onto the C57BL/6J genetic background (Eicher et al. 1982). When this same Ypos chromosome is backcrossed onto the DBA/2J or 129/Sv genetic backgrounds, testicular function is unaffected and normal ratios of male and female progeny are obtained (Eicher et al. 1982; Nallaseth 1987). Testicular dysfunction may result from improper reception by (C57BL/6J) autosomal loci of trans-activating testis-determining signals from the Tdy-locus of Ypos chromosomes (Eicher et al. 1982). However, some of the molecular analyses of genomic DNAs from female offspring of C57BL/6J Ypos males provide an alternative explanation. It is shown here that random mutations due to a generalized loss of control of recombination apparently result in the observed testicular dysfunction in C57BL/6J Ypos mice. Most genotypic and phenotypic traits characteristic of hybrid dysgenesis in other species (Kidwell et al. 1977; Hinton 1979; Bregliano et al. 1980; Bingham et al. 1982; Yannopoulos et al. 1987) are also observed in (M. musculus × M. poschiavinus) F1 hybrids and in various lines derived from them (Gropp et al. 1972; Cattanach and Moseley 1973; Capanna 1982; Eicher et al. 1982; Magnuson et al. 1985; Nallaseth 1987). These data collectively argue that the C57BL/6J Ypos mouse represents the first case of mammalian hybrid dysgenesis recognized at the level of molecular resolution.
Material and Methods

Mice

XX* males (C57BL/6J genetic backgrounds) and pure strain C57BL/6J and 129/Sv were obtained from Jackson Laboratories. 129/Sv Y^Pos and C57BL/6J Y^Pos males were initially transferred from the colonies of J. Barry Whitney III to the colonies at the University of South Carolina. _Mus poschiavinus_ (Valende) and _M. domesticus_ (Centreville Light) were provided by Michael J. Potter.

Southern and Densitometric Analysis

Genomic DNAs were extracted from livers by using a procedure modified from published methods (Nallaseth and Dewey 1986; Nallaseth 1987). DNAs were digested as per instructions, with restriction endonucleases that are commercially available. Digested DNAs were heat inactivated in 10 mM ethylene diaminetetraacetate (EDTA) and were ethanol precipitated, electrophoresed through (0.8%) agarose, 40 mM Tris, 20 mM sodium acetate, 1 mM EDTA pH 8.0, and 1 μg ethidium bromide/ml and were transferred either to nylon (N-Bond; Amersham) or, occasionally, to nitrocellulose (BA45; Schleicher and Schuell) filters. Hybridization and washing protocols are as described elsewhere (Nallaseth and Dewey 1986; Nallaseth 1987). Nomenclature of genomic clones and of the various Y-specific fragments subcloned from them are as described elsewhere (Nallaseth and Dewey 1986; Nallaseth 1987). EcoRI inserts of the Y-specific plasmid subclones were labeled either by replacement synthesis with T4 DNA polymerase or by nick-translation. (GATA)_5 oligonucleotides were end-labeled with T4 polynucleotide kinase. Some single-stranded probes of Y-specific sequences cloned into M13 were probes of choice. Details of choices of labeling protocols are as described elsewhere (Nallaseth 1987). Multiple exposures of autoradiographs were necessary for identifying and quantifying sequences that either (a) were 10-fold different in copy numbers or (b) because of nucleotide sequence mismatches annealed poorly with the probe. High- and low-level exposures of autoradiographs were attained by varying the following conditions: times and temperatures (-70°C or ambient) of exposure, use of intensifier screens, and preflashed X-ray films. XAR-5 (Kodak) X-ray films were developed in X-O-MAT (Kodak) processors. Restriction fragments of known and comparable copy numbers were routinely included as internal standards in densitometric quantifications. Densitometric analysis of X-ray films was performed in a Helena Laboratories densitometer, and the signal was analyzed with an Apple computer and Chromatochart programs (Nallaseth 1987).

Results

Locations of Y-linked Sequences Used as Probes, and Their Advantages for Detecting Aberrant Recombination Products

Because a generalized loss of genetic control of recombination affects multiple loci, noncoding repeated sequences are the probes of choice for identifying the resultant genotypic effects. An important and frequent technical complication of repeated sequence probes is the inability to detect minor aberrant recombination products in the presence of a vast excess of normal copies. Y-linked repeated sequences are sensitive probes because, owing to their male-limited haploidy and their location in the recombinationally suppressed chromosomal region, they allow the circumvention of this complication. Minor aberrant interchromosomal recombination products from the Y chromosome can be unambiguously detected in genomic DNAs of XX females.
probed with Y-linked sequences. In contrast, major aberrant recombination products can be unambiguously detected in XY individuals only when most copies of sequences hybridizing to these probes are either deleted or undergo mobility shifts. It is shown here that the probes used in the present study represent multiple non-pseudoautosomal loci; i.e., they are Y linked.

Several Y-specific EcoRI fragments are present at 90–170 copies/male genome (Nallaseth et al. 1983; Nallaseth and Dewey 1986; Nallaseth 1987). Sequences hybridizing to cloned copies of these fragments used as probes collectively represent a minimum of 15% of the murine Y chromosome (fig. 1A). This was determined by the product of the sizes of the inserts of genomic (lambda) clones and the copy numbers of the Y-specific probes subcloned from them. The sizes and the copy numbers of the major restriction fragments hybridizing to each of these Y-specific probes are listed in figure 1A. Each of these Y-specific EcoRI fragments cross-hybridized with 1–5 copies/haploid genome of autosomal or X-chromosomal EcoRI fragments (Nallaseth and Dewey 1986; Nallaseth 1987).

Three of these Y-specific probes—A, B, and C—failed to detect differences in restriction-endonuclease fragments between Y$^{Pos}$ chromosomes in feral Mus poschiavinus (Valende) males and 129/Sv Y$^{Pos}$ (backcrossed: N 18) males (Nallaseth 1987). This confirmed that the Y chromosomes in laboratory strains 129/Sv Y$^{Pos}$ and C57BL/6J Y$^{Pos}$ mice were derived from M. poschiavinus (Valende). These probes identified three distinct Y chromosomes in M. poschiavinus, M. domesticus (Centreville Light), and M. musculus (C57BL/6J) (Nallaseth 1987).

The Sxr region and the pseudoautosomal region are located, respectively, on the short arm near the centromere and on the long arm in the telomere of the murine Y chromosome (McLaren et al. 1988; Roberts et al. 1988) (fig. 1A). These two regions permit the cytogenetic localization of our probes. EcoRI-digested genomic DNAs from XX$^{Sxr}$ males were hybridized with probes A–C. They failed to detect Y-specific EcoRI fragments (data not shown). The expected cross-hybridizing X-linked fragments of probe A (Nallaseth and Dewey 1986; Nallaseth 1987) were clearly detected. These three Y-specific sequences were absent from the Sxr region. A fourth probe, (GATA)$_5$, identifies HaeIII or AluI fragments of $\sim$23 kb and $\geq$100 kb that are concentrated in the Sxr region and absent in XX females (Singh et al. 1984). Therefore, I monitored the structural integrity of the repetitive sequence fraction of the Sxr region by using the (GATA)$_5$ oligonucleotide (fig. 1A).

The frequency of X-Y exchange in the pseudoautosomal region is reflected as the frequency of females inheriting Y-chromosomal restriction fragments (Cooke and Smith 1986; Goodfellow et al. 1986; Harbers et al. 1986; Rouyer et al. 1986; Simmler et al. 1987). Y-chromosomal sequences hybridizing to probes A–C were tested for their frequency of transfer to X chromosomes of inbred strains. Major Y-specific EcoRI fragments of expected size (0.6 kb) and copy number ($\sim$100) were present (+++) in male genomic DNA (fig. 1B, lane 11) but absent (−) in all female genomic DNAs (fig. 1B, lanes 1–10) hybridized with probe A. Probe A also hybridized to several minor Y-specific EcoRI fragments (fig. 1B, lane 11). Although the background at 0.6 kb in one female genomic DNA (fig. 1B, in lane 10) was high, independent hybridizations have confirmed the absence of Y-specific EcoRI fragments in this DNA. Cross-hybridizing EcoRI fragments of 2.2 kb, 1.7 kb, 1.4 kb, and 0.8 kb were present in all female DNAs (fig. 1B, lanes 1–10). These fragments served as internal standards for probe A. After densitometric quantitation and correction for sizes of probes, the copy numbers of the 2.2-kb and 1.7-kb EcoRI fragments of probe A were estimated
to equal the unique-copy levels of the 12-kb EcoRI fragment of α-globin (fig. 1C, lanes 1 and 2). The other cross-hybridizing fragments of probe A were present at lower copy numbers and were detected at higher exposures.

Major Y-specific EcoRI fragments of expected sizes (4.3 kb and 3.7 kb) and copy numbers (~100) were present (++) in male genomic DNAs (fig. 1D, lanes 12 and 13) but absent (−) in female genomic DNAs (fig. 1D, lanes 1-11) hybridized with probe B. Probe B also hybridized to minor Y-specific EcoRI fragments of 2.2 kb and 6.6 kb (fig. 1D, lane 13). At high exposures, a unique-copy 3.9-kb EcoRI fragment cross-hybridizing with probe B was detected in all female DNAs (fig. 1D, lanes 1-11). This fragment served as the internal standard for probe B.

Major Y-specific EcoRI fragments of expected size (6.6 kb) and copy number (~100) were present (++) in feral male genomic DNA (fig. 1E, lane 10) but absent (−) in all female genomic DNAs (fig. 1E, lanes 1-9) hybridized with probe C. Probe C also hybridized to several minor Y-specific EcoRI fragments (fig. 1E, lane 10). The multicopy 1.8-kb EcoRI fragment of probe C was not detected, as it is an RFLP (restriction-fragment-length polymorphism) that is specific for the inbred-strain (C57BL/6J) Y chromosome. At high exposures, a unique-copy 3.5-kb EcoRI fragment cross-hybridizing with probe C was detected in all female genomic DNAs (fig. 1E, lanes 1-9). This fragment served as the internal standard for probe C.

An additional 36 sibling females also failed to reveal Y-specific fragments hybridizing to probes A-C (Nallaseth 1987). Thus, if, because of pseudoautosomal exchange, Y-specific sequences hybridizing to these three probes normally undergo transfer to karyotypically XX females, they do so at frequencies of <5% (P < 0.09). In addition, the X-linked fragments detected by probe A were monomorphic among C57BL/6J, in six other laboratory strains, and in M. poschiavinus and M. domesticus (Nallaseth 1987). The likelihood that these X-linked sequences were pseudoautosomal was further reduced when five additional restriction endonucleases failed to generate fragments characteristic of Y-chromosomal sequences (Nallaseth 1987). At least 200 independent females failed to show differences in these X-linked sequences, illustrating their extreme stability.

Considerable (>90%) sequence identity existed between these Y-linked fragments and published Y-specific sequences known to be distributed along the length of the Y chromosome (author's unpublished data). These results showed that the most likely location of the Y-linked repeated sequences hybridizing to probes A–C was in the entire, recombinationally suppressed Y-chromosomal region between the Sxr region and the pseudoautosomal region (fig. 1A). Therefore, the suitability of probes A–C for the experiments presented below was well established.

Variable Amounts of Ypos-specific Sequences in Female Sibling Progeny of C57BL/6J Y pos Males

Previous karyotypic analysis had failed to identify gross structural alterations of the Ypos chromosome in C57BL/6J Ypos mice (Eicher et al. 1982). However, others had reported that control of segregation and recombination of autosomes and X chromosomes was disrupted in mice derived from M. musculus × M. poschiavinus hybrids (Gropp et al. 1972; Cattanach and Moseley 1973; Capanna 1982; Magnuson et al. 1985). The possibility of structural instability of the Ypos chromosome in C57BL/6J Ypos mice was, therefore, pursued with the more sensitive approach of Southern analysis with Y-linked probes. As predicted by the instability of the Ypos chromosome, genomic DNAs from female progeny of C57BL/6J Ypos males contained variable amounts of
### Major Y-Chromosome Sequences

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>EcoR I</th>
<th>Hae III, Alu I</th>
<th>copies / chrom.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>pBC10-0.6</td>
<td>0.6</td>
<td></td>
<td>~90</td>
</tr>
<tr>
<td>B</td>
<td>pBC15-1.1</td>
<td>4.3, 3.7</td>
<td></td>
<td>~60, 30</td>
</tr>
<tr>
<td>C</td>
<td>pBA33-1.8</td>
<td>6.7, 1.8</td>
<td></td>
<td>~100 total</td>
</tr>
<tr>
<td>D</td>
<td>pBC10-0.17</td>
<td>0.169</td>
<td></td>
<td>~90</td>
</tr>
<tr>
<td></td>
<td>Sxr</td>
<td></td>
<td>&gt;100, ~23</td>
<td>&gt;1,000 [GATA]₅</td>
</tr>
</tbody>
</table>

- Y-chromosome (~40,000 kb)
- Sex Determining Region
  - [TDF, Sry, Sxr, and Tdy]
- Centromere
- Pseudoautosomal Region

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### Lanes

- **Type of Mouse DNA:**
  - Inbred Females (1-10)

- **Y-Specific Fragments:**
  - 2.2 kb
  - 1.7 kb
  - 1.4 kb
  - 0.8 kb
  - 0.6 kb
  - +++
Fig. 1.—Locations on Y chromosome and characterization of Y chromosome–linked probes. A, Schematic representation of mouse Y chromosome. The shaded areas, the hatched oval, and the blackened box represent the region of the Y chromosome over which X-Y interchromosomal recombination is suppressed. The superimposed hatched oval, the unblackened box, and the blackened box, respectively, the centromeric, pseudoautosomal, and Sxr regions. Listed above the Y chromosome are the Y-linked probes A–D and (GATA)$_3$, with their locations indicated by the shading and the blackened box, respectively. Sizes and estimated copy numbers of major Y-specific restriction fragments hybridizing to these probes are also listed above the Y chromosome. Fragments hybridizing to probe C include RFLPs characteristic of Y chromosomes from inbred strains. B and C. EcoRI-digested genomic DNAs (25 μg) from 10 females (lanes 1–10) and 1 male (lane 11) of the inbred strain C57BL/6J, hybridized with replacement synthesized insert of probe A (panel B). EcoRI-digested genomic DNAs (15 μg and 30 μg) from inbred strain females, simultaneously hybridized with nick-translated plasmids containing probe A sequences and the 3.2-kb Sall fragment of α-globin as inserts (panel C, lanes 1 and 2). Autoradiographs were exposed to high-intensity (panels B and C, lanes 1–9) and low-intensity (panel B, lanes 10 and 11; and panel C, lane 10) levels. Arrows identify identical restriction fragments that were electrophoresed on different gels. Major Y-specific EcoRI fragments and their estimated 100 copies are represented by triple-plus signs (++) when present and by a minus sign (−) when absent, below the lanes. D. EcoRI-digested genomic DNAs (25 μg) from 11 females of the inbred strain C57BL/6J (lanes 1–11) and 1 feral male (M; lanes 12 and 13), hybridized with nick-translated insert of probe B. Autoradiographs were exposed to high levels (lanes 1–12). Lane 13 is a low-level exposure of lane 12. Presence and absence of major Y-specific fragments of expected size and copy number are represented as in panels A and B. E. Filters represented in lanes 1–9 of panel D, stripped of probe and autoradiographed to ensure its complete removal. Both DNA on stripped filters and EcoRI-digested feral male (M) DNA on another filter were hybridized with the nick-translated insert of probe C (lanes 1–10). Autoradiographs were exposed to high-intensity (lanes 1–9) and low-intensity (lane 10) levels. The presence and absence of major Y-specific EcoRI fragments of expected 6.6-kb size and copy numbers are represented as in panels A and B.
Y-pos-specific sequences. They were detected by hybridization of DNAs with probes A–D and (GATA)$_3$.

The types of progeny resulting from the background on which the Y-pos chromosome was either structurally and functionally unstable (C57BL/6J) or stable (129/Sv) are schematically represented in figure 2. Four types of sibling C57BL/6J Y-pos females were classified on the basis of the estimated copy numbers of their Y-pos-specific sequence content relative to normal males. For simplification of analysis, the major Y-pos-specific sequence content of normal C57BL/6J Y-pos and 129/Sv Y-pos males hybridizing to probes A–D and (GATA)$_3$ was rounded to 100 copies, or 100%. The four types of C57BL/6J Y-pos females identified were High Copy Y, Low Copy Y, Traces Y, and Normal females, which had, respectively, equal amounts, 10%, 1%, and 0% of the Y-pos-specific sequence content of males (fig. 2). Some of the phenotypic and karyotypic characteristics of these females are also listed (fig. 2).

Detection of unique-copy levels or traces of Y-pos-specific sequences in female DNA with any one probe established Traces Y identity for that female. Regardless of the presence or absence of Y-pos-specific sequences hybridizing to the five probes in these analyses, these progeny are likely to be distinct from inbred strains. One example of their difference was the elevated frequency of transmission of a 4.7-kb EcoRI fragment cross-hybridizing with probe B. It was transmitted at six-fold higher frequencies to daughters of C57BL/6J Y-pos males than to daughters of inbred strain C57BL/6J males (author’s unpublished data). Molecular and genetic analysis of Traces Y and High Copy Y females are presented in the present paper.

Levels of Y-pos-specific Sequences Found in Traces Y Females

Transfer of unique-copy levels of Y-pos-specific sequences to autosomes or X chromosomes during meiosis is one result predicted by structural instability of the Y-pos chromosome. Genomic DNAs from sibling female progeny of C57BL/6J Y-pos...
males were tested for the presence of Y-pos-specific sequences by hybridization with probes A–C and (GATA)$_5$. Traces Y females and Normal females that were identified by this analysis are represented by T and F below their respective lanes in figure 3. XX$^{Sxr}$ and Normal male DNAs are represented by S and M below their respective lanes in figure 3. Estimated levels of Y-pos-specific restriction fragments identified in each DNA are listed below the respective lanes in figure 3.

Y-pos-specific EcoRI fragments hybridizing to probe A were of expected size (0.6 kb) and copy number (++) in male DNAs (fig. 3A, lanes 5 and 6) and absent (−) in Normal female DNAs (fig. 3A, lanes 3 and 4). X-linked internal standard EcoRI fragments were detected in all lanes (fig. 3A, lanes 1–6). The lower levels of the internal standards in male DNAs relative to female DNAs reflected the fivefold lower amounts of male DNAs that were loaded on the gel. Probe A hybridized to ~1.0 copy (+; see arrow in fig. 3A, lane 1) and ~0.1 copies (1/+; see arrow in fig. 3A, lane 2) of the Y-pos-specific 0.6-kb EcoRI fragment in two female DNAs and therefore identified them as Traces Y DNAs.

Y-pos-specific EcoRI fragments hybridizing to probe B were of expected size (4.3 kb, 3.7 kb, and 2.2 kb) and copy numbers (++) in male DNAs (fig. 3B, lanes 5–8) and were absent (−) in Normal female DNAs (fig. 3B, lanes 1, 2, and 4). The internal standard 3.9-kb EcoRI fragment for probe B was detectable in all female DNAs (fig. 3B, lanes 1–4). At the high exposure levels required to detect the 3.9-kb EcoRI fragment, Y-pos-specific EcoRI fragments in male DNAs hybridized to probe B were obscured. They were clearly visualized at lower exposure levels (fig. 3B, lanes 7 and 8). Probe B hybridized to unique-copy levels of the Y-pos-specific 4.3-kb EcoRI fragment in one female DNA (+; see arrow in fig. 3B, lane 3) and therefore identified it as Traces Y DNA.

Y-pos-specific EcoRI fragments hybridizing to probe C were of expected size (6.6 kb) and copy number (+) in mixtures of male/female DNAs (1/250) (fig. 3C, lane 9) and were absent (−) in normal female DNAs (fig. 3C, lanes 7 and 8). The internal standard 3.5-kb EcoRI fragment was detectable in all DNAs (fig. 3C, lanes 1 to 9). Probe C hybridized to unique-copy levels of the Y-pos-specific 6.6-kb EcoRI fragment in six female DNAs (+; see thick arrow in fig. 3C, lanes 1–6) and therefore identified them as Traces Y DNAs. A minor Y-pos-specific 9.7-kb EcoRI fragment of probe C was also detected in one Traces Y DNA (fig. 3C, lane 1).

HaeIII-digested DNA from females (fig. 3D, lanes 1–4 and 7–10), XX$^{Sxr}$ males (fig. 3D, lanes 5 and 11), and normal males (fig. 3D, lanes 6 and 12) were hybridized with end-labeled (GATA)$_5$ oligonucleotide. Autoradiographs of the same filter were exposed to high (fig. 3D, lanes 1–6) and low (fig. 3D, lanes 7–12) intensity levels. HaeIII fragments hybridizing to (GATA)$_5$ and specific for the Sxr region were of expected size (~23 kb and ≥100 kb) and total copy number (>1,000) in XX$^{Sxr}$ male DNAs (fig. 3D, lanes 5 and 11) and were absent in two Normal female DNAs (fig. 3D, lanes 1, 2, 7, and 8). Significantly lower copy-number levels of the ≥100-kb and ~23-kb HaeIII fragments hybridizing to (GATA)$_5$ were present in Normal male DNA (fig. 3D, lanes 6 and 12). The internal standard HaeIII fragments for (GATA)$_5$, ranging from ~5.0 kb to ~0.5 kb, were detected in all DNAs (fig. 3D, lanes 1–6 and 7–12). Relative to the total levels of ≥100-kb and ~23-kb HaeIII fragments in XX$^{Sxr}$ male DNA, the (GATA)$_5$ probe hybridized to unique-copy levels of the ~23-kb HaeIII fragment in two female DNAs (see arrow in fig. 3D, lanes 3 and 4) and therefore identified them as Traces Y DNAs. At the high levels of exposure required to detect these ~23-kb HaeIII fragments in Traces Y female DNAs, comigrating fragments in
Fig. 3.—\( Y^{pos} \)-specific sequence content of traces Y female (T) DNAs, shown to be 1% of that of male DNAs. Genomic DNAs were extracted from phenotypically Normal female (F) and male (M) offspring of C57BL/6J \( \times \) C57BL/6J \( Y^{pos} \) matings. Restriction-endonuclease digests and probes to which digested DNAs were hybridized are specified in panels A–E. \( Y^{pos} \)-specific restriction fragments and internal standards for each probe are specified in the text. Thick blackened arrows and unblackened arrows (in conjunction with individually specified lanes) represent, respectively, the presence and absence of major \( Y^{pos} \)-specific restriction fragments. The thin blackened arrows are used as in fig. 1. Autoradiographs were densitometrically quantitated and normalized to unique-copy-level internal standards identified in the text. \( Y^{pos} \)-specific sequences are represented, by symbols below the lanes, as estimated percentages or copies found in Normal males: + + + = 100% (100 copies); + = 10% (10 copies); + = 1% (1 copy); 1/ + = 0.1% (0.1 copy); and – = 0%. Normal and traces Y females and males identified by this analysis are represented, respectively, as F, T, and M, below the lanes. DNAs in lanes 1, 3, and 4 of panel A are, respectively, from the same females as are the DNAs in lanes 1, 2, and 3 of panel C. DNAs in lanes 1 and 2 of panel E are the same as DNAs in lanes 4 and 5 of panel E and in lanes 7, 8, 10, and 11 of panel E. A, EcoRI-digested genomic DNAs from females (25 \( \mu \)g; lanes 1–4) and males (5 \( \mu \)g; lanes 5 and 6), coelectrophoresed and hybridized with the replacement synthesized insert of probe A. Autoradiographs were exposed to high-intensity levels. B, EcoRI-digested genomic DNAs (25 \( \mu \)g) from females (lanes 1–4) and males (lanes 5–8), coelectrophoresed and hybridized with nick-translated insert of probe B. Lanes 1–6 were exposed to high-intensity levels; lanes 7 and 8 are low-level exposures of lanes 5 and 6. \( Y^{pos} \)-specific EcoRI fragments of expected size, as well as copy numbers for probe B, are
Fig. 3 (Continued) bracketed and listed to the right of the figure. The internal standard EcoRI fragment of 3.9 kb for probe B is identified to the left of the figure. C, Female genomic DNAs (25 μg; lanes 1–8) and mixtures of male/female genomic DNAs (0.1 μg/25 μg; lane 9), digested with EcoRI and hybridized with nick-translated insert of probe C. DNAs in lanes 1–9 were coelectrophoresed. D, Female DNAs (15 μg, lanes 1–4 and 7–10), pooled XX<sup>Str</sup> male (S) DNAs (15 μg; lanes 5 and 11), and male DNA (15 μg; lanes 6 and 12), digested with HaeIII, coelectrophoresed, and hybridized with end-labeled (GATA)<sub>3</sub> oligonucleotide. The same filter was autoradiographed to high-intensity (3 d at room temperature; lanes 1–6) and low-intensity (6–8 h at room temperature; lanes 7–12) levels. E, EcoRI-digested genomic DNAs (15 μg) from females (lanes 1 and 2) and a male (lane 3), hybridized with replacement synthesized insert of probe A. Both the filter autoradiographed in lanes 1 and 2 and the filter of male DNA (lane 3) hybridized with probe A were stripped of probe and rehybridized with nick-translated insert of probe C (lanes 4–6). Persistent residual signals, internal standards, and Y-specific EcoRI fragments are identified in the text. Genomic DNAs from the Traces Y female identified above (lanes 1 and 4) was digested with HaeIII. HaeIII-digested genomic DNAs (15 μg) from this Traces Y female (lanes 7 and 10), a Normal female (lanes 8 and 11), and a male (lanes 9 and 12) were coelectrophoresed and hybridized with end-labeled (GATA)<sub>3</sub> oligonucleotide. The same filter was autoradiographed to low-intensity (lanes 7–9) and high-intensity (lanes 10–12) levels of exposure.
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XXsry male and normal male (fig. 3D, lanes 5 and 6) DNAs were obscured. Both ≥100-kb and ~23-kb HaeIII fragments were clearly visualized at lower levels of exposure of this autoradiograph (fig. 3D, lanes 11 and 12). Comparison of the internal standard HaeIII fragments of (GATA)₅ in Normal female DNAs (fig. 3D, lanes 1, 2, 7, and 8) and in Traces Y female DNAs (fig. 3D, lanes 3, 4, 9, and 10) showed that all restriction-endonuclease digestions were complete and that more Normal female DNAs than Traces Y female DNAs were loaded on the gel. These results excluded trivial technical explanations such as partial digests and underloading, for the ~23-kb HaeIII fragment of (GATA)₅ being present in Traces Y female but absent in Normal female DNAs.

It was conceivable that, despite precautions, contamination by either plasmid or male genomic DNAs may be trivial technical explanations for the presence of traces of Y-specific sequences in female DNAs. Both of these potential explanations were ruled out by the data presented here. Some, but not all, of the Y-linked probes identified Y-pos-specific sequences in genomic DNA from the same Traces Y female. Genomic DNAs from the individual Traces Y females represented in lanes 1, 3, and 4 in figure 3A were, respectively, represented in lanes 1, 2, and 3 in figure 3C. Both probe A (fig. 3A) and probe C (fig. 3C) identified Y-pos-specific sequences in one of these females (fig. 3A, lane 1, and fig. 3C, lane 1). Although the respective internal standards of both probes A and C were clearly visible, only probe C revealed Y-pos-specific sequences in two of these females (compare fig. 3A, lanes 3 and 4, with fig. 3C, lanes 2 and 3). To further exclude contamination as a potential explanation, Traces Y genomic DNA on the same filter was successively hybridized with probes A and C. Genomic DNA from the same individual Traces Y female hybridized with probes A, C, and (GATA)₅ are represented in lanes 1, 4, 7, and 10 in figure 3E. Y-pos-specific sequences (see thick blackened arrow in fig. 3E) of probes A (fig. 3E, lanes 1 and 3) and (GATA)₅ (fig. 3E, lanes 7 and 10) of expected size and copy number were detected in this Traces Y female DNA. The filter represented in lanes 1 and 2 of figure 3E was stripped of probe, and both it and a similarly treated filter of male genomic DNA (fig. 3E, lane 3) were rehybridized with probe C (fig. 3E, lanes 4–6). Although the internal standards of probe C (3.5 kb) and probe A (residual signals at 2.2 kb and 1.7 kb) were clearly detected, the expected Y-pos-specific 6.6-kb EcoRI fragment hybridizing to probe C was absent in this Traces Y female DNA (see unblackened arrow in fig. 3E, lanes 4 and 6). Furthermore, the ~23-kb HaeIII fragment hybridizing to (GATA)₅ was present at unique-copy levels in this Traces Y female DNA (see thick blackened arrow in fig. 3E, lane 10) and was absent in Normal female DNA (fig. 3E, lane 11). The expected copy number of the ~23-kb HaeIII fragment was detected in coelectrophoresed male DNA (fig. 3E, lanes 9 and 12). Contamination by male DNA cannot explain detection of Y-pos-specific sequences in the same Traces Y DNA by only two of the three Y-linked probes. Since we have no plasmids of ~23-kb size, detection of ~23-kb HaeIII fragments in Y-pos-specific sequences hybridized with the (GATA)₅ oligonucleotide cannot be attributed to contamination by plasmids. In any case, most plasmid and other DNAs would be digested to small fragments, because of the high frequency of sites for HaeIII. This result also suggested the random representation of loci from the Y-pos chromosome hybridizing to probes A–C and (GATA)₅ in Y-pos-specific sequences of Traces Y females. Sequences from the entire length of Y-pos chromosomes were transmitted to 29%–83% of Traces Y female progeny per generation (table 1; Nallaseth 1987).

Karyotypic analysis of five independent Traces Y females that had 40 chromo-
somes failed to reveal a Y^pos chromosome, confirming that they were karyotypically XX. The presence of a Y^pos chromosome was confirmed in two of the male siblings of these females. In an identical mating, Eicher et al. (1982) reported 42 XX females.

Y^pos chromosomes from High Copy Y Females of N4 and N5 Generations

Sterile sex-reversed, karyotypically XY^pos (High Copy Y) females represent high proportions of N3 progeny of C57BL/6J Y^pos males (Eicher et al. 1982; Nallaseth 1987). However, extensive karyotypic analysis failed to reveal gross structural changes of the Y^pos chromosome from these High Copy Y females (Eicher et al. 1982; author's unpublished data). Mutations due to higher sequence instability in the testis-determining Sxr region, which represents only 0.05% of the Y chromosome, may explain this discrepancy. Although the testis-determining Tdy locus has been convincingly localized to the Sxr region of the murine Y chromosome (Cattanach et al. 1982; McLaren and Monk 1982; Singh and Jones 1982; McLaren et al. 1988; Roberts et al. 1988), fragments hybridizing specifically to the murine Tdy locus have not been cloned (Page et al. 1987; Koopman et al. 1989; Palmer et al. 1989). An alternative approach tested for higher instability of repeated sequences in Sxr regions than in non-Sxr regions of inactive Y^pos chromosomes. Copy numbers and ratios of restriction fragments from Sxr regions and non-Sxr regions were compared by hybridizing High Copy Y female DNAs with each of probes (GATA)_5 and A-D.

EcoRI-digested genomic DNAs from High Copy Y (fig. 4A, lanes 1 and 2) and Normal females (fig. 4A, lane 3) and from inbred strain (fig. 4A, lane 4), 129/Sv Y^pos (fig. 4A, lane 5), and C57BL/6J Y^pos (fig. 4A, lane 6) males were hybridized with probes A–C and rDNA. As expected, the rDNA-specific and Y^pos-specific EcoRI fragments were, respectively, present and absent in Normal female DNA. The multicopy 1.8-kb EcoRI fragment hybridizing to probe C was an RFLP that was characteristic of inbred strain (C57BL/6J) Y chromosomes. It was present at unique-copy levels in DNA from males with the Y^pos chromosome and in DNA from High Copy Y females. This confirmed the identity of the Y^pos chromosome in these females. In these High Copy Y female and male genomic DNAs, there were no differences in the mobilities of major EcoRI fragments hybridizing to probes A–C and rDNA (fig. 4A). Y^pos-specific EcoRI fragments from 31 High Copy Y female DNAs and from one of each of the three types of listed male DNAs were densitometrically quantitated, and their ratios were plotted as per the legend to figure 4C. Values of hybridization ratios of seven pairs of Y^pos-specific EcoRI fragments hybridizing to probes A–C (fig. 4A and fig. 4C, lower panel) did not differ significantly (>2.5 X) between either male or inbred-strain-male DNAs and the mean for High Copy Y female DNAs (fig. 4C, upper panel). Furthermore, in High Copy Y female DNAs, the range and the mean of the hybridization ratios of each of these seven pairs of Y^pos-specific EcoRI fragments also did not differ significantly (>2.5 X) (fig. 4C, lower panel).

Probe D, however, which also hybridized to sequences from the non-Sxr region, identified maximal sequence instability of Y^pos chromosomes. All ~100 copies of Y^pos-specific EcoRI fragments (fig. 5B, lanes 1 and 5), hybridizing to probe D shifted from 169 bp (see unblackened arrow in fig. 5B, lane 2) to 225 bp (see blackened arrow in fig. 5B, lane 2) in one High Copy Y female DNA. Only partial shifts were detected in High Copy Y female sibling DNAs (fig. 5B, lanes 2–4), in male DNAs (fig. 5B, lane 5), and in 27 other High Copy Y female DNAs, also from N4 and N5 generations.
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(data not shown). To accentuate instability of sequences hybridizing to probe D, only genomic DNAs displaying maximal 225-bp/169-bp hybridization ratios were densitometrically quantitated and plotted as per the legend to figure 4C. The mean of the hybridization ratios for probe D fragments in High Copy Y female DNAs (≥8.9) was 11–22-fold higher than their hybridization ratios in male DNAs (0.4 and 0.8) (fig. 4C, upper panel). In High Copy Y female DNAs the hybridization ratios for probe D fragments was 0.1–26, or 260-fold, which was 29-fold higher than their mean hybridization ratios (~8.9) (fig. 4C, lower panel).

However, only one of eight pairs of Y-pos-specific EcoRI fragments from the non-Sxr region displayed this high degree of instability. The remaining seven pairs of Y-pos-specific EcoRI fragments were stable in 31 High Copy Y female DNAs from three types of tissue (kidney, liver, and spleen) and from two backcross generations. Thus, non-Sxr regions of inactive Y-pos chromosomes from High Copy Y females of N4 and N5 generations displayed great stability during both meiotic and somatic cell division.

In contrast, the Sxr regions of inactive Y-pos chromosomes from the aforementioned High Copy Y females were significantly unstable. Genomic DNAs from these High Copy Y females (fig. 4B, lanes 1, 2, 4, and 5), a Normal female (fig. 4B, lane 3), a Normal N3 male (fig. 4B, lane 7), and XX^Sxr males (fig. 4B, lane 6) were digested with either HaeIII (fig. 4B) or AluI (data not shown) and hybridized with the end-labeled eicosanucleotide (GATA)₅. Internal standards showed that digests were complete, and equal amounts of DNA were loaded. Significant variations in copy numbers and hybridization ratios of the ≥100-kb/~23-kb HaeIII (fig. 4B) and AluI (data not shown) fragments hybridizing to (GATA)₅ were detected within High Copy Y female DNAs and between High Copy Y female, male, and pooled XX^Sxr male DNAs (see arrows in fig. 4B, lanes 1–7). The ≥100-kb and ~23-kb HaeIII and AluI fragments hybridizing to (GATA)₅ were densitometrically quantitated and plotted as per the legend to figure 4C. XX^Sxr males have only ~0.05% of the Y chromosome including the Tdy locus (Cattanach et al. 1982; Evans and Burtenshaw 1982; Singh and Jones 1982). However, in pooled XX^Sxr male genomic DNAs, the copy number of the ≥100-kb HaeIII fragments was at least 11-fold higher than that in genomic DNAs from High Copy Y females (fig. 4B, lanes 1, 2, 4, and 5) and males (fig. 4B, lane 7). The mean of the hybridization ratios of ≥100-kb/~23-kb HaeIII and AluI fragments hybridizing to (GATA)₅ in High Copy Y female genomic DNAs was ~3.7 (fig. 4C, upper panel). It did not differ significantly from the hybridization ratios of the ≥100-kb/~23-kb HaeIII fragments of (GATA)₅, either in pooled XX^Sxr male genomic DNAs (~3.8) or in genomic DNAs from an N3 male (~1.97) (fig. 4C, upper panel). In contrast, in High Copy Y female genomic DNAs, the hybridization ratios of ≥100-kb/~23-kb HaeIII and AluI fragments of (GATA)₅ were 166.5 and 100, respectively (fig. 4C, lower panel). These ranges were, respectively, 45-fold and 51-fold higher than the mean hybridization ratios of these HaeIII and AluI fragments in High Copy Y female genomic DNAs. Phenotypic inactivity of Sxr regions of Y-pos chromosomes in High Copy Y females correlates well with the molecular instability of their repeated sequence content. It may be that the high variability and the copy-number reductions of sequences in the Sxr regions relative to sequences in the non-Sxr regions reflect the indirect selection for analysis of inactive Y-pos chromosomes, i.e., High Copy Y females.
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Hybridization Ratio (range)

Hybridization Ratio (Mean)

FIG. 4.—High Copy Y females (HC) of N4 and N5 backcross generations whose YPos chromosomes were more extensively altered in Sxr regions than in non-Sxr regions. Types of matings and identities of symbols or abbreviations are as in the legend to fig. 3. A. EcoRI-digested genomic DNAs (20 µg) from High Copy Y females (lanes 1 and 2), a Normal female (lane 3), and males (lanes 4-6), coelectrophoresed and hybridized with probes A-C and with ribosomal DNA (rDNA) as internal standard. High Copy Y females were from the N4 and N5 backcross generations. Males were of the inbred strain C57BL/6J (lane 4), 129/Sv YPos (lane 5), and C57BL/6J YPos (lane 6). Major Y-specific EcoRI fragment sizes—and probes to which they hybridized—are listed to the right. Autoradiographs were exposed to intermediate-intensity levels. Lower exposures of autoradiographs with a linear range of signals were densitometrically quantitated to provide the data plotted in panel C. B. HaeIII-digested genomic DNAs (10 µg) from some of High Copy Y females (lanes 1, 2, 4, and 5), Normal females (lane 3), XX'Y males (pooled DNAs; lane 6), and a Normal N3 male (lane 7), hybridized with end-labeled (GATA)5 oligonucleotide. DNAs in lanes 1 and 2, lanes 3-5 and lanes 6 and 7 were coelectrophoresed. Autoradiographs were exposed to low-intensity levels (6-8 h at room temperature). These and other autoradiographs were densitometrically quantitated for the data plotted in panel C. C. rDNA-specific and YPos-specific EcoRI fragments of rDNA, probes A-C (panel A and fig. 5A) and probe D (fig. 5B), densitometrically scanned. Areas below peaks were integrated, and the area for each YPos-specific EcoRI fragment was normalized to the area for the 12-kb EcoRI fragment of rDNA (internal standard). Sxr region specific ≥100 kb and ~23 kb HaeIII (panel B) and AluI (data not shown) fragments hybridizing to (GATA)5 were also densitometrically scanned. Areas below these two peaks were integrated and normalized to integrated areas below the fragments from ~5.0 kb to ~0.5 kb, which served as internal standards for the (GATA)5 probe. Hybridization ratios of pairs of restriction fragments identified on the X-axis of the lower graph of panel C were calculated for each of the genomic DNAs identified in the upper graph of panel C. The mean of hybridization ratios of pairs of fragments was determined for High Copy Y female DNAs and for all male DNAs. The range from minimal to maximal hybridization ratios of pairs of fragments was determined for High Copy Y female DNAs. The polymorphic 1.8-kb EcoRI fragment hybridizing to probe C was only quantitated in DNA from inbred-strain males. Probes A-D were absent in XX'Sxr male DNA. All DNAs, unless stated otherwise, were extracted from livers. Probes A-D were hybridized to 31 DNAs from N4 and N5 HC-Y female siblings and to 1 DNA from an N3 male parent; three types of tissues (kidney, liver, and spleen) from each of eight High Copy Y females plus one type of tissue from seven High Copy Y females totaled 31 DNAs. (GATA)5 was hybridized to 15 DNAs from N4 and N5 High Copy Y female siblings and from 1 DNA from an N3 male parent; two types of tissue (kidney and liver) from each of seven High Copy Y females plus one type of tissue from 1 High Copy Y female totaled 15 High Copy Y DNAs. □ = C57BL/6J (XY B6) ♦; □ = C57BL6J YPos (XY Pos) ♦; □ = 129/Sv YPos (XY Pos) ♦; □ = Sxr (XX'Sxr) ♦; and ■ = High Copy Y (XY Pos) ♦.

Restriction Analysis: Exclusion of Mechanisms of Extrachromosomal Transmission of YPos-specific Sequences

One explanation for the origin of YPos-specific sequences in Low Copy Y and Traces Y females is extrachromosomal transmission via circular elements, retrottransposons, or transposons. One common feature to all three of these mechanisms of
Fig. 5.—Precise mobility shifts of all copies of multicopy Y Pos-specific EcoRI fragments in DNAs from High Copy Y females. Types of matings and identities of symbols and abbreviations are as in figs. 3 and 4. A, EcoRI-digested genomic DNAs (25 μg) from Normal female (lane 1), High Copy Y females (lanes 2–4) and male (lane 5) coelectrophoresed and hybridized with rDNA and with probes A–C. High Copy Y females were siblings of the N5 generation. The expected sizes and identities of the EcoRI fragments hybridizing to each probe are listed on the right and left, respectively. B, Filter autoradiographed for panel A, after being stripped of probes and rehybridized with replacement synthesized insert of probe D. Probe D identifies ~100 copies of a Y Pos-specific EcoRI fragment of 169 bp.

extrachromosomal transmission is the random loss or gain of restriction fragments at the sites of excision and/or reintegration, at high frequencies.

More than 90% of High Copy Y, Low Copy Y, and Traces Y females failed to reveal Y Pos-specific EcoRI fragments of unexpected sizes when hybridized with probes A–C. Those High Copy Y and Traces Y female DNAs that displayed EcoRI fragments of expected size were further analyzed with different enzymes or their various combinations, so that there were a total of 17 distinct types of digests. Digests were designed to either release or retain genomic sequences flanking Y Pos-specific sequences hybrid-
izing to each probe in High Copy Y, Low Copy Y, and Traces Y females. Enzymes with four-base and six-base recognition sites were included (Nallaseth 1987). Randomization of restriction fragments characteristic of induction of retroviruses (Jenkins and Copeland 1985) or transposons (Bingham et al. 1982) and excision into extrachromosomal sequences (Smith and Vinograd 1972; Stanfield and Lengyel 1979; Calabreta et al. 1982; Krolewski et al. 1982; Potter 1984; Walbot and Cullis 1985) were not detected. Thus, it was concluded that these mechanisms do not account for generation of Y^Pos-specific sequences in these three types of females. Some of the remaining \(\sim 10\%\) of these female DNAs that displayed Y^Pos-specific EcoRI fragments of unexpected sizes that hybridized to probes A–C were analyzed below.

Two distinct mechanisms for losses of Y^Pos-specific EcoRI fragments were defined by Traces Y and High Copy Y female DNAs. One of the effects on High Copy Y DNA further excluded extrachromosomal transmission of Y^Pos-specific sequences as a possible mechanism.

Major Y^Pos-specific EcoRI fragments of expected size (0.6 kb) and copy number (\(\sim 100\)) were present (++) in \(M.\ poschiavinus\) (Valende) male genomic DNA hybridized with probe A (fig. 6, lane 7). Characteristic minor Y^Pos-specific EcoRI fragments (4.2 kb and 3.8 kb) were also present at \(\sim 10\)-fold lower copy number in \(M.\ poschiavinus\) (Valende) male genomic DNA hybridized with probe A. Both major and minor Y^Pos-specific EcoRI fragments hybridizing to probe A remained absent (-) from Normal female genomic DNAs, even at high exposures (fig. 6, lanes 1 and 5). Internal standard EcoRI fragments (2.2 kb, 1.7 kb, and 0.8 kb) hybridizing to probe A were present in all DNAs and showed that restriction-endonuclease digestions of genomic DNAs were complete. Y^Pos-specific EcoRI fragments of the expected size of 0.6 kb were detected in two Traces Y female genomic DNAs hybridized with probe A (see thick blackened arrows in fig. 6, lanes 4 and 6). However, in genomic DNAs from two Traces Y female siblings hybridized with probe A, an EcoRI fragment of the unexpected size of 5.4 kb was present at unique-copy levels (see blackened arrows in fig. 6, lanes 2 and 3). Both 0.6-kb and 5.4-kb EcoRI fragments were present in genomic DNA from a third Traces Y female hybridized with probe A (see blackened arrows in fig. 6, lane 4). Overexposure of autoradiographs of \(M.\ poschiavinus\) (Valende) male genomic DNA hybridized with probe A failed to reveal a similar sized (5.4-kb) EcoRI fragment (data not shown). Therefore, the 5.4-kb EcoRI fragments were reproducibly generated during transfer of Y^Pos-specific sequences to autosomes/X chromosomes transmitted to two Traces Y female siblings (fig. 6, lanes 2 and 3). Because double crossovers are rare events, at least one of the restriction sites flanking the 0.6-kb EcoRI fragment hybridizing to probe A must have been lost during transfer of Y^Pos-specific sequences.

In contrast to the above loss of restriction sites of a single copy fragment, precise mobility shifts of all \(\sim 100\) copies of Y^Pos-specific restriction fragments were detected in genomic DNAs from some High Copy Y females. These High Copy Y females were also from the N5 backcross generation. The multicopy Y^Pos-specific sequences hybridizing to probes A and D were well defined by restriction endonuclease mapping and sequencing. The Y^Pos-specific 600-bp EcoRI fragment of probe A and the Y^Pos-specific 169-bp EcoRI fragment of probe D were contiguous and present at the frequency of once per \(\sim 14\)-kb \(HpaI\) repeat unit. Thus, these two probes hybridized to sequences spanning (\(\sim 14\) kb \(\times\) \(\sim 100\) copies \(\cong\)) 1,400 kb of the Y^Pos chromosome. Approximately 50\% of the sequences within the 169-bp EcoRI fragment of probe D
Fig. 6.—Reproducible losses of EcoRI restriction sites of Y-pos-specific sequences in Traces Y female DNAs. Types of matings and identities of symbols and abbreviations are as in fig. 3. EcoRI-digested genomic DNAs (25 μg) from Normal females (lanes 1 and 5), Traces Y females (lanes 2–4 and 6), and a Mus poschiavinus (Valende) male (lane 7) were hybridized with the replacement synthesized insert of probe A. The DNAs in lanes 1–3 and in lanes 4–6 were extracted from siblings and were coelectrophoresed. Autoradiographs were exposed to high-intensity (lanes 1–6) and low-intensity (lane 7) levels. Sizes of EcoRI fragments hybridizing to probe A are listed to the right.

consists of the 3’ terminus of rodent Alu (B1) sequences and their oligo A tail (Nallaseth 1987).

Expected sizes and copy numbers of EcoRI fragments hybridizing to probes A–C and rDNA were present in genomic DNAs from three High Copy Y female siblings (fig. 5A, lanes 2–4) and from males (fig. 5A, lane 5). Y-pos-specific EcoRI fragments were absent from Normal female DNAs (fig. 5A, lane 1). The filter was stripped of probes and rehybridized with probe D (fig. 5B). Probe D hybridized to ~100 copies of a 169-bp EcoRI fragment that was present in male DNA (fig. 5, lane 5) but absent in Normal female DNA (fig. 5B, lane 1). In genomic DNA from one High Copy Y female sibling, all ~100 copies of the Y-pos-specific EcoRI fragments hybridizing to probe D had precisely shifted from 169 bp (see unblackened arrow in fig. 5B, lane 2) to 225 bp (see blackened arrow in fig. 5B, lane 2). Partial shifts were detected in genomic DNAs of sibling High Copy Y females (fig. 5B, lanes 3 and 4) and in males (fig. 5B, lane 5). Thus, these mobility shifts were due to single-generation events, either during development in the (N4) paternal germ line or in the (N5) somatic cells.
in High Copy Y females. Precise mobility shifts of the \( Y^{pos} \)-specific 169-bp \( EcoRI \) fragment occurred at the high frequency of 8 of 48 \( XY^{pos} \) offspring from the backcross generations N3–N7. Restriction analysis showed that these mobility shifts were limited to the 169-bp \( EcoRI \) fragment within the \( \sim 14 \)-kb \( HpaI \) repeat unit (author's unpublished data).

Trivial technical explanations such as partial digests or aberrant electrophoresis cannot explain either the reproducibility and precision or the limitation of mobility shifts to one of five multicopy \( EcoRI \) fragments within a single lane. The molecular mechanisms causing mobility shifts must operate coordinately, precisely, and selectively on each of \( \sim 100 \) copies of the 169-bp \( EcoRI \) fragment interspersed over \( \geq 1,400 \) kb of the \( Y^{pos} \) chromosome. Because all other \( Y^{pos} \)-specific repeats were intact and the mobility shifts were limited to 169 bp within the \( \sim 14 \)-kb \( HpaI \) repeat unit, intrachromosomal events rather than extrachromosomal transmission are the likely mechanisms. These two results showed that deletions and / or rearrangements of \( Y^{pos} \) chromosomes were likely to be associated with the transfer of \( Y^{pos} \)-specific sequences to the autosomes or \( X \) chromosomes that were transmitted to Traces \( Y \) females.

Sequences First Deleted and Transmitted from \( Y^{pos} \) Chromosomes during Meiosis in \( F1 \) Males

The C57BL/6J and 129/Sv genetic backgrounds are, respectively, inductive and noninductive for dysfunction of \( Y^{pos} \)-encoded testis determination. A correlation has been detected among the presence in sibling females of traces of \( Y^{pos} \)-specific sequences and intact inactive \( Y^{pos} \) chromosomes, the selective instability of repeated sequences in the \( Sxr \) region, and nonfunctional testis determination. To test whether the aberrant recombinational mechanisms that randomly delete \( Y^{pos} \)-specific sequences from \( Y^{pos} \) chromosomes actually correlate with the disruption of testis determination, a genetic approach was taken. The \( Y^{pos} \) chromosome was backcrossed from the 129/Sv to the C57BL/6J genetic backgrounds, where it was structurally and functionally destabilized at high frequencies. If the transmission of \( Y^{pos} \)-specific sequences to XX females (i.e., as deletion products) and the functional inactivity of \( Y^{pos} \) chromosomes in their \( XY^{pos} \) female siblings result from related mechanisms, then four predictions can be made for successive backcross generations (N). First, traces of \( Y^{pos} \)-specific sequences should not be detected on the 129/Sv genetic background on which the testis-determining \( Sxr \) region is fully functional. Second, traces of \( Y^{pos} \)-specific sequences should not be detected in somatic tissues of \( F1 \) hybrids of C57BL/6J \( \times \) 129/Sv \( Y^{pos} \). Third, traces of \( Y^{pos} \)-specific sequences should first be detected in backcross generations preceding the backcross generation in which inactive \( Y^{pos} \) chromosomes of High Copy \( Y \) females are detected. Fourth, generating of traces (i.e., deletions) should have a cumulative effect on the functional inactivity of a population of \( Y^{pos} \) chromosomes in successive backcross generations. These four predictions of a relationship between the generation of \( Y^{pos} \)-specific trace sequences (i.e., deletion products) and the functional inactivation of the \( Sxr \) region on the \( Y^{pos} \) chromosome were examined and found to be correct. They are addressed below.

To minimize the presence of non-129/Sv sequence content, a male of the 129/Sv \( Y^{pos} \) (N18) strain was used to produce (C57BL/6J \( \times \) 129/Sv \( Y^{pos} \)) \( F1 \) hybrid offspring. Genomic DNAs from \( F1 \) hybrid females were analyzed for their \( Y^{pos} \)-specific sequence content, and their sibling \( F1 \) hybrid males were mated with pure-strain C57BL/6J females. Genomic DNAs of \( \left[ C57BL/6J \times F1 \right. \) (C57BL/6J \( \times \) 129/Sv \( Y^{pos} \))] \( N2 \) female offspring were analyzed for their \( Y^{pos} \)-specific sequence content.
Similarly, a backcross series was independently initiated with a mating of C57BL/6J × C57BL/6J Y\sup{pos} (N3), and genomic DNAs of N4–N7 female offspring were analyzed for their Y\sup{pos}-specific sequence content. The results of these matings are shown in figures 3–8, and they are summarized in table 1.

Genomic DNAs from an inbred strain male (fig. 7A, lane 12, fig. 7B, lane 10) and female (fig. 7A, lane 11, and fig. 7B, lane 9), hybridized with probe A, displayed Y-specific and cross-hybridizing EcoRI fragments of expected sizes and copy numbers. The cross-hybridizing EcoRI fragments of probe A were clearly detected in genomic DNAs from F1 females (fig. 7A, lanes 1–10). However, the Y\sup{pos}-specific 0.6-kb EcoRI fragment hybridizing to probe A was absent from these F1 female DNAs (fig. 7A, lanes 1–10). Similarly, the respective presence and absence of cross-hybridizing and Y-specific EcoRI fragments of probe C in F1 female genomic DNAs was established (data not shown). Therefore, sequences from Y\sup{pos} chromosomes were not transferred to either autosomes or X chromosomes in germ lines of 129/Sv Y\sup{pos} males. Furthermore, cross-hybridizing sequences of probes A and C were not amplified and rearranged so as to acquire electrophoretic mobilities of Y\sup{pos}-specific sequences during the somatic development of F1 progeny.

High frequencies (75%; table 1) of genomic DNAs from N2 females hybridized with probe A (see thick arrow in fig. 7B, lanes 1–8) revealed unique-copy levels of the Y\sup{pos}-specific 0.6-kb EcoRI fragment. Genomic DNAs of these N2 females hybridized with probe C and (GATA)\textsubscript{3} also revealed unique-copy levels of Y\sup{pos}-specific fragments (data not shown). Therefore, Y\sup{pos}-specific sequences were first transferred to autosomes or X chromosomes in germ lines of F1 males.

Genetic heterozygosity was sufficient—and predominant proportions of C57BL/6J alleles were therefore not required—for generating traces of Y\sup{pos}-specific sequences (i.e., deletion products). Genetic heterozygosity of C57BL/6J genetic backgrounds was not sufficient for generating High Copy Y females which were first detected in N3 generations. Thus, the second and third predictions of a causal relationship between the generating of Y\sup{pos}-specific deletion products and functional inactivity of Y\sup{pos} chromosomes were observed to be true.

The frequencies of Traces Y females containing Y\sup{pos}-specific sequences in the backcross generations N2, N4, N5, and N6 were, respectively, 75%, 52%, 29%, and 39% (table 1). A χ² analysis (χ² = 50.05) with 1 degree of freedom showed a significant probability (P < 0.001) that these traces of Y-specific sequences would be transmitted to XX females at frequencies compatible with normal pseudoautosomal exchange. In contrast, the frequencies of High Copy Y females in the backcross generations N2, N4, N5, and N6 respectively, were 0%, 14%, 49%, and 31% (table 1). Deleting Y\sup{pos}-specific sequences from a population of Y\sup{pos} chromosomes apparently had a cumulative effect on the inactivation of their testis-determining (Sxr region) functions in subsequent backcross generations. Thus, the fourth prediction of a causal relationship also held true.

The absolute requirement for the germ-line presence of a functional Y\sup{pos} chromosome for the transmission of traces of Y\sup{pos}-specific sequences to female offspring was also established. Female progeny of (C57BL/6J Y\sup{pos} Normal females (N4) × C57BL/6J pure-strain males) matings lacked any Y\sup{pos}-specific sequences (table 1; Nallaseth 1987). The absence of Y\sup{pos}-specific sequences in these Normal female (N5) progeny further excluded the possibility that amplifications and rearrangements of the cross-hybridizing sequences of Y-specific probes would allow them to acquire Y\sup{pos}-specific mobilities.
FIG. 7.—Trace levels of Y-pos-specific sequences first generated during meiotic division in F1 males. Identities of symbols and abbreviations are as in figs. 3 and 4. EcoRI-digested genomic DNAs (25 μg) from (C57BL/6J × C57BL/6J 129/Sv Ypos) F1 females (panel A, lanes 1–10), (C57BL/6J × C57BL/6J 129/Sv Ypos) N2 females (panel B, lanes 1–8), C57BL/6J pure-strain females and males (panel A, lanes 11 and 12, and panel B, lanes 9 and 10) were hybridized with the replacement synthesized insert of probe A. Genomic DNAs in panel A, lanes 1–10 in panel A, lanes 11 and 12, and in panel B, lanes 1–8, and in panel B, lanes 9 and 10, were coelectrophoresed. Sibling males of F1 females were mated with inbred-strain C57BL/6J females to obtain N2 female offspring.
FIG. 8.—Precise structural alterations of stable multicopy Y<sup>pos</sup>-specific EcoRI fragments in DNAs of High Copy Y females from terminal backcross generations (N6 and N7). Symbols and abbreviations are as in figs. 3 and 4. K, L, and S denote lanes showing DNAs from kidney, liver, and spleen, respectively, of individual High Copy Y females. Sibling males of High Copy Y females of the N5 generation (analyzed in fig. 4A–C) sired High Copy Y and Normal female siblings of the N6 generation (analyzed in this fig.). Sibling males of High Copy Y females (analyzed in this fig.) sired High Copy Y female siblings of the N7 generation (analyzed panel C). All XY<sup>pos</sup> offspring in N7 litters were sterile. A and B, EcoRI-digested genomic DNAs (20 μg) from a Normal female (panels A and B, lanes 1) and High Copy Y female siblings from the N6 generation (panels A and B, lanes 2–7) were coelectrophoresed and hybridized either with probe C (panel B) or with rDNA and probes A–C (panel A). Autoradiographs were exposed to low-intensity (panel A) and high-intensity (panel B) levels. Identities and sizes of Y<sup>pos</sup>-specific and rDNA-specific EcoRI fragments are listed to the left and right, respectively, of panel A and to the right of panel B. C, Genomic DNAs extracted from livers of High Copy Y female siblings of N7 generation. EcoRI-digested genomic DNAs (20 μg) were coelectrophoresed and hybridized with probes A–C. Autoradiographs were exposed to low-intensity levels.

Stable Repeated Sequences on Y<sup>pos</sup> Chromosomes Showing Precise Structural Changes in Terminal Backcross Generations (N6 and N7)

The stability of a single population of Y<sup>pos</sup> chromosomes was followed over the four backcross generations N4–N7, by hybridization of High Copy Y female genomic DNAs with probes A–D (figs. 4, 6, and 8 and table 1). All XY<sup>pos</sup> progeny in the N7 generation were sterile, thus ending this mouse line. Densitometric analysis of genomic DNAs from High Copy Y females of N4 and N5 generations showed that Y<sup>pos</sup>-specific EcoRI fragments hybridizing to probe D and to probes A–C were, respectively, unstable and stable during germ-line and/or somatic development (figs. 4A and 4C and 6). The mean hybridization ratios of two major Y<sup>pos</sup>-specific EcoRI fragments (4.3 kb and 3.7 kb) hybridizing to probe B was 2.0 (fig. 4C). This hybridization ratio was
### Table 1
Types of C57BL/6J Ypos Females and Their Frequencies/Generation

| PARENTAL MATING | % OF FEMALE PROGENY (C57BL/6J Ypos) | NO. OF FEMALES ASSAYED (no. of females excluded)
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>NORMAL* (F)</td>
<td>TRACES Y</td>
</tr>
<tr>
<td>C57BL/6J (P) × 129/Sv Ypos (6)</td>
<td>F1</td>
<td>100</td>
</tr>
<tr>
<td>C57BL/6J (P) × F1 Ypos (6)</td>
<td>N2</td>
<td>25</td>
</tr>
<tr>
<td>C57BL/6J (P) × N3 Ypos (6)</td>
<td>N4</td>
<td>14*</td>
</tr>
<tr>
<td>C57BL/6J (P) × N4 Ypos (6)</td>
<td>N5</td>
<td>17*</td>
</tr>
<tr>
<td>C57BL/6J (P) x N5 Ypos (6)</td>
<td>N6</td>
<td>0*</td>
</tr>
<tr>
<td>× C57BL/6J Ypos (F) (N4) (9)</td>
<td>N5</td>
<td>100</td>
</tr>
</tbody>
</table>

**NOTE.**—All female offspring of C57BL/6J (P) × C57BL/6J Ypos (6) matings are classified as C57BL/6J Ypos females. Ypos = Y chromosome of *Mus musculus* on different genetic backgrounds.

* Progeny in which Ypos-specific sequences were not detected with the three moderately repeated Y-specific probes denoted as F.

* Not counted in the number of females assayed.

* Three or four probes present at trace levels; fourth probe present at low copy levels.

* Data from Normal (or Traces Y) females in which the presence or absence of Ypos-specific sequences was not conclusively established are omitted.

* Ypos = Y chromosome of C57BL/6J strains.

Invariant (<0.5 ×) in 31 genomic DNAs extracted from three types of tissue (kidney, liver, and spleen) from these High Copy Y females and from males with either Ypos chromosomes or inbred strain Y chromosomes.

However, EcoRI-digested genomic DNAs from High Copy Y females of the N6 and N7 generation hybridized with probe B showed that the ratio of the 4.3-kb and 3.7-kb fragments was altered >30-fold. All copies of the major 3.7-kb and minor 2.2-kb Ypos-specific EcoRI fragments hybridizing to probe B were clearly absent in spleen DNA—but not in kidney or liver DNAs—from one High Copy Y female sibling of the N6 generation (see unblackened arrows in fig. 8A, lane 7). All other major Ypos-specific EcoRI fragments hybridizing to probes A–C were identical in kidney, liver, and spleen DNAs from both N6 siblings (fig. 8A, lanes 2–6). Losses of these two fragments occurred in one of four and two of three High Copy Y females of the N6 and N7 generations, respectively. Although genomic DNA in lane 2 of figure 8A was smeared, major Ypos-specific EcoRI fragments hybridizing to probes A–C were visible. In addition, ratios of the rDNA-specific (12-kb) EcoRI fragment to each of the major Ypos-specific EcoRI fragments of probes A–C differed significantly (i.e., more than fivefold) between genomic DNAs from High Copy Y females of the N6 generation (fig. 8A, lanes 2–6) and N4 and N5 generations (fig. 4A).

The filter that was autoradiographed for figure 8A was previously probed with probe C and exposed to high levels (fig. 8B). Minor Ypos-specific 180-bp EcoRI fragments hybridizing to probe C were present in liver DNAs (see blackened arrow in fig. 8B, lanes 3 and 6) but were absent in kidney and spleen DNAs (fig. 8B, lanes 2, 4, 5, and 7) from both of these High Copy Y female siblings of the N6 generation.

The filter autoradiographed for figure 8A was stripped of probe and reannealed with probe D. All copies of the Ypos-specific 169-bp EcoRI fragment hybridizing to probe D displayed mobility shifts from 169 bp to 110 bp in liver DNAs—but not in
kidney and spleen DNAs—from both of these High Copy Y female siblings of the N6 generation (data not shown).

In EcoRI-digested genomic DNAs from livers of two High Copy Y female siblings of the N7 generation, the major 3.7-kb and the minor 2.2-kb fragments hybridizing to probe B were absent in one (see unblackened arrows in fig. 8C, lane 2) but were present in the other (fig. 8C, lane 1). All other major Y-Pos-specific EcoRI fragments hybridizing to probes A–C were indistinguishable in DNAs from these two N7 siblings (fig. 8B, lanes 1 and 2).

Therefore, multicopy Y-Pos-specific EcoRI fragments hybridizing to each of the three probes B–D underwent distinct types of precise structural changes in different tissues. The Y-Pos chromosome was structurally destabilized during the somatic development of High Copy Y females. Trivial technical explanations for these mobility shifts included either partial and star activity of restriction endonucleases or aberrant electrophoretic conditions. Star activity was tested for and was empirically excluded; furthermore, these explanations were incompatible both with the precision and reproducibility of mobility shifts between different genomic DNAs and with their limitation to one or two of five multicopy Y-Pos-specific EcoRI fragments of DNA within a single lane.

Therefore, eventually, even stable repeated sequences on Y-Pos chromosomes showed precise structural alterations at high frequencies. These Y-Pos chromosomes were structurally destabilized in terminal backcross generations, suggesting that their maintenance on C57BL/6J backgrounds had some cumulative effect.

Discussion

Structural Destabilization: Functional Inactivation of Feral Y-Pos Chromosomes on C57BL/6J Inbred Backgrounds

The data presented in the present paper demonstrated that the Y-Pos chromosome from the feral species Mus poschiavinus was structurally destabilized at high frequencies when it was backcrossed onto the C57BL/6J inbred genetic background. However, the Y-Pos chromosome was stable on the 129/Sv inbred genetic background. Molecular and genetic analysis showed (a) that there was a strong correlation between the structural instability of the Y-Pos chromosome on C57BL/6J backgrounds and eventual functional inactivation of its Tdy locus and (b) that the Y-Pos chromosome functioned normally on 129/Sv genetic backgrounds (fig. 2).

Two results predicted by the structural destabilization of Y-Pos chromosomes were confirmed, by using Y-linked multicopy sequences, by probing genomic DNAs from daughters of C57BL/6J Y-Pos males. In genomic DNAs from XX females, unique-copy levels or traces of Y-Pos-specific sequences were present at high frequencies (29%–75% /Y-chromosomal repeat sequence/generation). These were Traces Y females. In genomic DNAs from some of their XY-Pos female siblings, multiple copies of Y-Pos-specific restriction fragments either were absent or underwent precise coordinated mobility shifts. These were High Copy Y females with structurally destabilized Y-Pos chromosomes. Trivial technical explanations for trace sequences in Traces Y females and for destabilized Y-Pos chromosomes in High Copy Y females were excluded. Data for Low Copy Y females are not presented.

In 80 N4 offspring from matings that were identical to those in the present study, Eicher et al. (1982) identified 42 XX females, 16 XY-Pos females, and 22 XY-Pos with ovaries and/or ovotestis. Five Traces Y and 2 High Copy Y females were karyotypically XX and XY-Pos, respectively (author's unpublished data). Given that 11 of 21 N4
females analyzed in the present work were Traces Y females, at least some of the XX females in the study by Eicher et al. (1982) were also Traces Y females. Inability to detect either presence of YPos chromosomal fragments or gross structural changes of YPos chromosomes in karyotypes of XX and XYPos females could be explained by two results from this work: (1) in High Copy Y females of the N4 or N5 generation, repeated sequence copy numbers and stabilities were more extensively reduced in Sxr regions containing the Tdyl locus and representing <0.05% of YPos chromosomes than in non-Sxr regions; and (2) since fragments <200 kb cannot be visualized in optical microscopes, both Traces Y and Low Copy Y females would escape detection.

Although it would be ideal to show that deletions/rearrangements of the Tdyl locus inactivate testis-determining functions of YPos chromosomes, current understanding of this complex developmental pathway (Singh et al. 1984; Chandra 1985; Page et al. 1987; Koopman et al. 1989; Palmer et al. 1989; Schneider-Gädicke et al. 1989), precludes such an unambiguous correlation. Instead, when a genetic approach was used, increased structural instability indirectly correlated with the functional inactivity of a population of YPos chromosomes backcrossed from 129/Sv onto C57BL/6J genetic backgrounds for seven successive generations. Since all XYPos offspring were sterile, this line ended in the N7 generation. Traces Y females were absent in F1 but were present at high frequencies in litters from N2 and subsequent generations. High Copy Y females were absent in N2 but appeared at increasing frequencies in the subsequent, N3–N7 generations. YPos-linked repeated sequences that were stable during both germ-line and somatic development in the N4 and N5 generations underwent precise structural destabilization at high frequencies during somatic development in the N6 and N7 generations. Thus, maintenance of YPos chromosomes on C57BL/6J backgrounds resulted in structural destabilization, which eventually inactivated functions of the Tdyl and other loci. The inactivation of testis-determining functions of the YPos chromosome because of its sequence instability is predictably stochastic. Therefore, the mouse line being derived will end in sterile individuals after varying numbers of backcrosses of the YPos chromosome onto the C57BL/6J genome. By selecting for males lacking any overt external signs of sex reversal, it has been possible to slightly extend the number of backcross generations to N10, before the line ends in sterile XYPos individuals (J. Barry Whitney III, personal communication). Others (Nagamine et al. 1987; Biddle and Nishioka 1988) have reported similar extensions of numbers of backcrosses of YPos chromosomes onto C57BL/6J genetic backgrounds. However, the results presented in the present paper make it likely that C57BL/6J YPos mice in each of these laboratories is genotypically distinct.

Stable X-linked transmission of YPos-specific sequences from the germ line of a Low Copy Y female mated with males of an inbred strain was tested for. Nontransmission of YPos-specific sequences from the Low Copy Y female may be explained by one of the following three observations: (1) There were 10-fold differences of copy numbers of YPos-specific sequences in three tissues of this female. Their complete absence from ovaries may explain nontransmission of YPos-specific sequences by this Low Copy Y female. (2) The results in the present paper showed that sequences on YPos chromosomes underwent structural destabilization during germ-line and somatic development. Their excision from chromosomes of developing ova or embryos may explain nontransmission of YPos-specific sequences from this Low Copy Y female. (3) Finally, other donor sequences or transgenes are also not transmitted from male (Wilkie and Palmiter 1987) and female (Rohan et al. 1990) germ lines of transgenic mice. In the case of the male mouse, the MyK 103 transgene had to be excised during
spermatogenesis (Wilkie and Palmiter 1987) to allow formation of viable sperm. Instability of other nucleotide sequences during somatic or embryonal development has also been reported (Kelly et al. 1989; Rohan et al. 1990).

Loss of Genetic Regulation of Recombination: Possible Cause of Instability of \( Y^{\text{Pos}} \) Chromosomes

Locus-specific and chromosome-specific genetic regulation of the five fundamental functions of eukaryotic chromosome biology—i.e., replication, repair, recombination (interchromosomal and intrachromosomal), condensation, and segregation—are well established in yeast, \textit{Neurospora}, and \textit{Drosophila} (Baker et al. 1976; Gatti et al. 1980; Strathern et al. 1981; Klahr (1985); Surosky and Tye 1988). Recombination in all organisms is known to be under rigorous genetic regulation (Kucherlapati and Smith 1988). The inbred strain C57BL/6J is genetically distinct from both 129/Sv and most other inbred strains (Taylor 1972; Cattanach and Moseley 1973; Forejt and Iványi 1975; Fitch and Atchley 1985). It is likely that alleles controlling recombination in C57BL/6J and 129/Sv inbred strains are distinct, thus explaining the instability of \( Y^{\text{Pos}} \) chromosomes on C57BL/6J but not on 129/Sv genetic backgrounds.

At least two distinct types of aberrant recombination events on \( Y^{\text{Pos}} \) chromosomes were identified by \( Y^{\text{Pos}}\)-specific sequences in Traces Y and High Copy Y females. \( Y^{\text{Pos}}\)-specific sequences in Traces Y females were first transmitted from F1 male germ lines. They were only partially representative of copies (\( \sim 1/100 \)) and types [A–C and (GATA)\(_5\)] of interspersed repeated sequences from the entire length of \( Y^{\text{Pos}} \) chromosomes. Losses of flanking restriction sites of these traces of \( Y^{\text{Pos}} \)-specific sequences were also detected. These characteristics of \( Y^{\text{Pos}} \)-specific sequences are inconsistent with translocations, extrachromosomal elements, and recombinational hot spots. They are consistent with random interchromosomal recombination products which require limited sequence homology for heteroduplex formation, e.g., gene conversions. The inferred mechanism for the transfer of \( Y^{\text{Pos}} \)-specific sequences to XX females is the loss of suppression of \( X^B-Y^{\text{Pos}} \) interchromosomal recombination. This inference is supported by other observations on X-Y recombination. X-Y interchromosomal pairing is known to be destabilized in offspring from matings between laboratory strains and wild mice (Cattanach and Moseley 1973; Forejt and Iványi 1975; Matsuda et al. 1983; Handel 1987). Many translocations from Y chromosomes are to X chromosomes (Singh and Jones 1982; Page et al. 1987; Petit et al. 1987), and unequal crossovers have been shown to occur in the murine \textit{pseudoautosomal} region (Harbers et al. 1986).

\( Y^{\text{Pos}} \)-specific sequences in XY \( Y^{\text{Pos}} \) females showed contrasting characteristics. Precise reproducible losses or mobility shifts of all or most copies of multicopy \( Y^{\text{Pos}} \)-specific restriction fragments occurred in a single generation during somatic and/or germ-line development. These losses and mobility shifts were restricted to specific interspersed repeated sequences and to specific restriction fragments within a repeated sequence. Because of the high precision and frequency of these events and because of the large chromosomal sizes represented by interspersed repeated sequences, these were highly coordinated mechanisms. As somatic X-Y pairing is absent in mammals, these events must involve either intrachromosomal mechanisms of recombination or some other sequence modification.

The loss of control of recombination, which resulted in sequence instability, was not limited to combinations of the C57BL/6J inbred strain and feral \( Y^{\text{Pos}} \) chromosomes. Y chromosomes from the inbred strain C57BL/6J and another feral mouse,
Instability and Inactivation of Murine Y Chromosomes

M. domesticus (Centreville Light), were reciprocally backcrossed for two generations. Traces of Y-specific sequences and precise losses and mobility shifts of multicopy Y-linked sequences also resulted from both types of these matings (author's unpublished data).

The high frequencies and precision of highly coordinated changes of sequences on Y^pos^ chromosomes suggest that these changes were likely to result from mechanisms that were distinct from other mechanisms causing genetic instability. The loss of epigenetic control of retroviral replication by a maternal resistance factor and FV restriction loci in SWR/J × RF/J murine hybrids results in the induction of retroviruses (Jenkins and Copeland 1985). Similarly, their lower frequencies and different types of aberrant recombination products suggest that the meiotic instability of the polymorphic repeat sequence PR 1 and VrDNAs in the murine rDNA loci (Kuehn and Arnheim 1983; Kominami et al. 1985), microrecombinations in the murine major histocompatibility locus (Geliebter and Nathenson 1988), somatic instability of murine variable numbers of tandem repeats (VNTRs) (Kelly et al. 1989), and the rearrangement-induced premeiotically (RIP) in N. crassa (Selker et al. 1987) are mechanistically unrelated to the instability of the Y^pos^ chromosome.

It is now recognized that unknown mechanisms result in two opposing effects on the repeated sequence fraction of the mammalian genome. Although there is a large extent of nucleotide sequence flux within it, structural and functional order are imposed on the mammalian genome (Goldman et al. 1984; Dover and Flavell 1984; Bernardi et al. 1985; Dover and Tautz 1986; Schmid and Shen 1986; Zuckerlandl 1986; Dover 1987, 1990; Korenberg and Rykowski 1988; Kucherlapati and Smith 1988). Fluxes of repeated sequences include such large genome-encompassing effects as their concerted evolution, retrotranspositioning (Dover and Flavell 1984; Baltimore 1985; Rogers 1985; Dover and Tautz 1986; Schmid and Shen 1986; Zuckerlandl 1986; Dover 1987, 1990), and expansions and contractions of VNTRs (Dover 1987, 1990; Jeffreys et al. 1990). The rate, precision, and extent of these nucleotide sequence fluxes cannot be explained either by Mendelian inheritance or by stochastic, slow, and unregulated processes such as drift, selection, and "selfishness" of DNA (Dover and Tautz 1986; Schmid and Shen 1986; Dover 1987, 1990). Loss of regulation of any of the unconventional mechanisms, either imposing structural and functional order on mammalian genomes or driving large nucleotide sequence fluxes through them, are potential mechanisms for the non-Mendelian transmission of Y^pos^-specific sequences documented in the present paper.

Loss of Y^pos^ Chromosome Structure and Function as Consistent with Hybrid Dysgenesis Occurring in Mammals

When genomes of two evolutionarily diverged species are hybridized, a collection of genotypic and phenotypic dysfunctions are induced. Traits collectively characterizing hybrid dysgenesis are requirements of directionality of genetic crosses, induction of transposons, disruption of regulation of interchromosomal (meiotic) and intrachromosomal (mitotic) recombination, suppression of recombination at active loci, induction of recombination in inactive loci (and genomes), chromosome nondisjunction, segregation (or transmission-ratio) distortion, gonadal dysfunction (sterility), high frequencies of mutations, and embryonal lethalties (Haldane 1922; Dobzhansky 1951; Tracey 1972; Forejt and Iványi 1975; Kidwell et al. 1977; Bregliano et al. 1980; Bingham et al. 1982; Handel 1987; Yannopoulos et al. 1987). Most of the genotypic and phenotypic traits defining hybrid dysgenesis in other species are also identified in
the C57BL/6J Y<sup>Pos</sup> mouse. Two of the most important traits characterizing hybrid dysgenesis are loss of control of recombination and testicular dysfunction, and they are specifically recognized in C57BL/6J Y<sup>Pos</sup> mice (for a list of these and other traits, see table 2). Many of these traits were also reproduced when the Y chromosome from another feral species, <i>M. domesticus</i> (Centreville Light), was backcrossed onto C57BL/6J inbred strains (author's unpublished results).

Like most inbred strains, C57BL/6J originated as a hybrid of <i>M. musculus domesticus</i> ♀ × <i>M. m. musculus</i> ♂ (Bishop et al. 1985). The C57BL/6J inbred-strain genome contains alleles that make it distinct from all other inbred strains (Taylor 1972; Cattanach and Moseley 1973; Forejt and Iványi 1975; Bonhomme et al. 1984; Fitch and Atchley 1985). The Y<sup>Pos</sup> chromosome itself originates from <i>M. poschiavinus</i> species, which is highly diverged from all laboratory strains, both in its karyotypic constitution (Gropp et al. 1972; Cattanach and Moseley 1973; Capanna 1982) and in its nuclear (Cattanach and Moseley 1973) and mitochondrial (Ferris et al. 1983) genomes. It is likely that (a) differences in C57BL/6J alleles controlling recombination and (b) the evolutionary divergence of Y<sup>Pos</sup> chromosomes act together to produce sequence instability of the Y<sup>Pos</sup> chromosome in this inbred strain.

### Table 2

**Traits Characterizing Dysgenesis in Strains Derived from <i>Mus musculus</i> × <i>M. poschiavinus</i> Hybrids**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotypic:</strong></td>
<td></td>
</tr>
<tr>
<td>Directionality of genetic crosses: C57BL/6J♂ × 129/Sv Y&lt;sup&gt;Pos&lt;/sup&gt;♂ causes dysgenesis</td>
<td>Present study</td>
</tr>
<tr>
<td>Induction of interchromosomal recombination: Y-linked sequences are transferred to other chromosomes (transferred sequences are derived from B1, R, and satellite repeats) and sequences from &gt;6,000 kb are transferred from Y&lt;sup&gt;Pos&lt;/sup&gt; chromosome</td>
<td>Nallaseth 1987; present study</td>
</tr>
<tr>
<td>Suppression of interchromosomal recombination in autosomal and X chromosomal loci</td>
<td>Cattanach and Moseley 1973</td>
</tr>
<tr>
<td>Aneuploidy of autosomes and X chromosomes</td>
<td>Gropp et al. 1972; Cattanach and Moseley 1973; Capanna 1982; Magnuson et al. 1985</td>
</tr>
<tr>
<td>Increased frequency of transmission of 4.7-kb EcoRI autosomal/X chromosomal unique sequence</td>
<td>Nallaseth 1987</td>
</tr>
<tr>
<td>Induction of mitotic instability of Y&lt;sup&gt;Pos&lt;/sup&gt; chromosomes</td>
<td>Nallaseth 1987</td>
</tr>
<tr>
<td>High frequency of mutations</td>
<td>Cattanach and Moseley 1973</td>
</tr>
<tr>
<td><strong>Phenotypic:</strong></td>
<td></td>
</tr>
<tr>
<td>Disruption of primary sex determination</td>
<td>Eicher et al. 1982; present study</td>
</tr>
<tr>
<td>Disruption of oogenesis and spermatogenesis</td>
<td>Gropp et al. 1972; Cattanach and Moseley 1973; Capanna 1982; Eicher et al. 1982; Magnuson et al. 1985; Nallaseth 1987</td>
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<tr>
<td>Disruption of embryogenesis</td>
<td>Gropp et al. 1972; Cattanach and Moseley 1973; Magnuson et al. 1985</td>
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<td>Disruption of secondary masculinization</td>
<td>Eicher et al. 1982; Nallaseth 1987</td>
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</table>
Two important differences are noted between the hybrid dysgenesis detected in C57BL/6J Y°OS mice and that in Drosophila (Kidwell et al. 1977; Bregliano et al. 1980; Bingham et al. 1982; Yannopoulos et al. 1987). First, transposon (or retroviral) excision does not explain the presence of Y°OS-specific sequences in females. Second, in addition to random recombination, precise, reproducible, and high-frequency sequence instabilities are induced de novo in loci that are normally recombinationally suppressed on the Y chromosome during hybrid dysgenesis. Only imprecise recombination at chromosome breaks marking sites of transposon excision occurs during hybrid dysgenesis in Drosophila. Finally, since natural hybrid zones of M. musculus × M. poschiavinus have been identified (Gropp et al. 1972; Capanna 1982), these observations may have important implications for speciation both in these hybrids and in laboratory strains, which have originated as hybrids (Bishop et al. 1985).

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LITERATURE CITED


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