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Deletion Analysis of the Selfish B Chromosome, Paternal Sex Ratio (PSR), in the Parasitic Wasp Nasonia vitripennis

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ABSTRACT

Paternal Sex Ratio (PSR) is a “selfish” B chromosome in the parasitoid wasp Nasonia vitripennis. It is transmitted via sperm, but causes supercondensation and destruction of the paternal chromosomes in early fertilized eggs. Because this wasp has haplodiploid sex determination, the effect of PSR is to convert diploid (female) eggs into haploid (male) eggs that carry PSR. Characterizing its genetic structure is a first step toward understanding mechanisms of PSR action. The chromosome is largely heterochromatic and contains several tandemly repeated DNA sequences that are not present on the autosomes. A deletion analysis of PSR was performed to investigate organization of repeats and location of functional domains causing paternal chromosome destruction. Deletion profiles using probes to PSR-specific repetitive DNA indicate that most repeats are organized in blocks on the chromosome. This study shows that the functional domains of PSR can be deleted, resulting in nonfunctional PSR chromosomes that are transmitted to daughters. A functional domain may be linked with the \( \text{psr22} \) repeat, but function may also depend on abundance of PSR-specific repeats on the chromosome. It is hypothesized that the repeats act as a “sink” for a product required for proper paternal chromosome processing. Almost all deletion chromosomes remained either functional or nonfunctional in subsequent generations following their creation. One chromosome was exceptional in that it reverted from nonfunctionality to functionality in one lineage. Transmission rates of nonfunctional deletion chromosomes were high through haploid males, but low through diploid females.

A variety of genetic elements gain transmission advantage relative to their associated genome. Such elements have been referred to as meiotic drive genes (Sandler and Novitski 1957; Crow 1979; Lyttle 1991), selfish or parasitic genes (Östergren 1945; Nur 1966, 1977; Doolittle and Sapienza 1980; Orgel and Crick 1980; Werren, Nur and Wu 1988) or ultra-selfish genes (Crow 1988; Wu and Hammer 1991). Examples include Segregation Distorter (Hartl and Hiraizuma 1976; Temin et al. 1991) and Sex-Ratio (James and Jaenike 1990) in Drosophila and \( t \)-alleles in Mus (Silver 1985; Klein 1986; Lyon 1989).

Because meiotic drive is often an aberrant form of basic developmental processes, drive systems provide useful models in the study of meiosis and gametogenesis (Wu and Hammer 1991). For example, chromosomes carrying the Segregation Distorter complex in Drosophila (Peacock and Miklos 1973) and the \( t \)-locus in Mus (Olds-Clarke and Peitl 1985; Zeitz and Bennett 1985) were found to cause dysfunction of sperm carrying the nondriving homologue. Current studies on the mechanisms of these systems contribute to our understanding of chromosome inactivation, chromosome condensation and sperm maturation (e.g., Brown et al. 1989; Wu and Hammer 1991; Howard et al. 1990; Uehera et al. 1990; Powers and Ganetsky 1991; Temin 1991). Moreover, molecular analysis of the chromosomal regions that cause drive can be informative about evolutionary processes at the level of DNA organization and chromosome structure.

Paternal Sex Ratio (PSR) is a driving chromosome with an unusual form of transmission (Werren, Nur and Eckbush 1987; Nur et al. 1988; Werren 1991). PSR is a supernumerary (or B) chromosome found in the parasitoid wasp Nasonia vitripennis that causes all-male offspring. The PSR chromosome is only transmitted via sperm and causes supercondensation and subsequent loss of the paternal chromosomes, except itself, in fertilized eggs. Because Nasonia has haplodiploid sex determination, the effect of PSR is to convert diploid eggs, which would normally develop into females, into haploid eggs that develop into PSR-bearing males. PSR is transmitted to the next generation through the sperm of those males and it again eliminates the paternal chromosomes (which were maternally derived from the previous generation). Because PSR completely eliminates the genome of its “host”, it is the most extreme example of a selfish

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PSR is a small submetacentric chromosome that is mostly or completely composed of heterochromatin (Nur et al. 1988). It is estimated to comprise 5–8% of the haploid genome. Molecular analysis has revealed that PSR contains four families of tandemly repeated DNA sequences (Nur et al. 1988; Eickbush, Eickbush and Werren 1992). Repeat families are distinguished based upon sequence differences and lack of cross-hybridization under standard stringency. Three major families (psr2, psr18 and psr22) are specific to the PSR chromosome, a fourth (psr79) is enriched on PSR but also present on the autosomes at lower abundance. The psr18 family can be subdivided into four repeat types (psr10, psr13, psr18 and psr105). In addition, repetitive DNAs have been found on the autosomes that are not present on PSR (i.e., NV85, NV104 and NV126; Eickbush, Eickbush and Werren 1992).

Characterizing the genetic structure of the PSR chromosome is a first step toward understanding the genetic and molecular basis of its action. Because PSR recognizes and selectively destroys the paternal chromosomes, it is a potential system for studying mechanisms of chromosomal imprinting and condensation, as well as early mitosis in the fertilized egg. Here we present a deletion analysis of PSR. The main objectives are: (1) determine whether nonfunctional PSR chromosomes can be generated by deletion, (2) determine the organization and localization of repeats on the chromosome, and (3) look for possible functional domains (i.e., specific regions on the chromosome associated with PSR function).

MATERIALS AND METHODS

Culturing Nasonia: Nasonia vitripennis is a 2–3 mm sized parasitoid wasp that lays its eggs in pupae of blowflies and fleshflies (Whitting 1967). It can easily be maintained in the laboratory (see Whitting (1967) Werren (1991) and Beukeboom and Werren (1992) for details on biology and culturing methods). Generation time is 14 days at 25°C. The PSR chromosome was routinely maintained in the MI strain (Macomb, Ill.; Saul et al. 1965) and is indicated as PSR (MI). This strain carries the Maternal Sex Ratio (MSR) distorter, which causes females to fertilize 90–100% of eggs (Skinner 1982). This is convenient for PSR maintenance, because PSR is only transmitted to fertilized eggs via sperm. In parasitoid wasps, males are normally derived from unfertilized eggs.

Generating deletions: Two methods were used to create deletions in the PSR chromosome: irradiation (IR) and cytoplasmic incompatibility (CI). Irradiation: PSR males were irradiated as pupae (9 days old) or as adults (14 days old) with gamma (cobalt) radiation at varying doses (3–20K rads). Spermatogenesis in Nasonia takes place in the pupal stage and is completed upon emergence (Hogge and King 1975). By utilizing males of different ages, it was possible to examine the effect of irradiation at different spermatogonial stages. Irradiated PSR males typically produced all-male progeny (as do wild-type PSR males). F1 males were crossed to virgin females from the MI strain. After mating, F1 males were screened for the presence of PSR and for deletions by DNA hybridizations with PSR-specific probes (see screening for PSR). The effects of deletions on PSR action could be detected in the F2 generation by whether the chromosome was transmitted to male progeny (indicating PSR action) or female progeny (loss of action) (Figure 1).

Cytoplasmic incompatibility: The second method for creating PSR deletion chromosomes made use of cytoplasmic incompatibility. In some crosses between strains of Nasonia, the paternal chromosomes are fragmented and destroyed due to the presence in the egg of cytoplasmic microorganisms (Ryan and Saul 1968; Breeuwer and Werren 1990). However, centromere containing fragments occasionally survive and are transmitted at low frequency (Ryan, Saul and Conner 1985, 1987). Therefore, cytoplasmic incompatibility can be used to create deletions in the PSR chromosome. Indeed, incompatible crosses between standard PSR(MI) males and t277 females resulted in all-male families and survival of the PSR chromosome at low frequency (25%). Moreover, the surviving PSR chromosomes often contained deletions which could be tested for loss or retention of PSR function (Figure 1).

Screening for PSR: Because wasps carrying the PSR chromosome are morphologically indistinguishable from noncarriers, molecular assays and progeny testing were used to screen for the presence of PSR. Transmission of PSR to F1 progeny in the IR and CI analysis was determined by dot-blotting homogenate of single males and hybridizing with a "PSR-cocktail" probe (containing repeat types psr2, psr10, psr18 and psr22). Similarly, testing for transmission from F1 to F2 was done by probing 5 pooled F2 progeny of each sex.

Dot-blot assay: The PSR chromosome was detected by hybridizing total wasp DNA to PSR specific probes. Radio-
active probes were prepared from lambda clones containing seven different repetitive DNAs specific to PSR (NUR et al. 1988; EICKBUSH, EICKBUSH and WERREN 1992). 

The autosomal NV126 probe was used as control for homogenization efficiency in a dot-blot assay. Data are from EICKBUSH, EICKBUSH and WERREN (1992).

Deletions in the PSR chromosome were detected by hybridizing DNA of individual carrier males to radioactive labeled probes from lambda clones containing seven different repetitive DNAs specific to PSR. DNA homogenates from single wasps carrying a normal ("wild-type") PSR chromosome were used to make dilution series (1, 1/2, 1/4, 1/8 and 1/16X). Two such dilution series were used as reference on each filter, one in the top row and one in the bottom row (Figure 2). In either row each dilution was dotted in duplicate. DNA solutions from wasps carrying PSR deletion chromosomes were dotted in duplicate in the remaining rows. This provided an internal control on hybridization intensity differences due to dotting variability. Finally, some homogenates from known carrier and noncarrier individuals were always dotted onto the corners of each filter to verify the PSR specificity of the assay. One copy of each filter was then hybridized to one of the eight probes.

A deletion profile of a PSR chromosome was obtained by comparing hybridization intensities of the sample dots to each probe with the reference dots of known intensity (Figure 2). Comparisons of hybridization intensities of sample and reference dots were restricted to within filters. Therefore, lack of linearity in the response range of the film. A 50% difference in signal intensity could reliably be scored by eye as revealed after checking with a density scanner. Duplicate dots rarely differed in signal intensities. If they did, such homogenates were dotted and hybridized again.

An autosomal repeat NV126 (EICKBUSH, EICKBUSH and WERREN 1992) was used as control for amount of DNA loaded on the filters. Noncarrier controls always had full hybridization to the autosomal NV126 probe, but never gave any signal when hybridized to PSR specific repeats. The only exception was the psr79 repeat, which is also present on the autosomes (EICKBUSH, EICKBUSH and WERREN 1992). Noncarrier controls did hybridize to the psr79 repeat, but at intensities much lower (usually < 1/16, sometimes 1/16) than observed in PSR males.

TABLE 1

<table>
<thead>
<tr>
<th>Repeat family</th>
<th>Repeat type</th>
<th>Repeat length (bp)</th>
<th>Miscellaneous</th>
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<tbody>
<tr>
<td>psr2</td>
<td>psr2</td>
<td>171</td>
<td>Specific to PSR</td>
</tr>
<tr>
<td>psr18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>psr10</td>
<td>psr10</td>
<td>207</td>
<td>Cross-hybridize even</td>
</tr>
<tr>
<td>psr105</td>
<td>psr105</td>
<td>214</td>
<td>at high stringency</td>
</tr>
<tr>
<td>psr13</td>
<td>psr13</td>
<td>212</td>
<td>Cross-hybridize even</td>
</tr>
<tr>
<td>psr18</td>
<td>psr18</td>
<td>215</td>
<td>at high stringency</td>
</tr>
<tr>
<td>psr22</td>
<td>psr22</td>
<td>183</td>
<td>Specific to PSR</td>
</tr>
<tr>
<td>psr79</td>
<td>psr79</td>
<td>94</td>
<td>Present on PSR, and on autosomes at lower abundance</td>
</tr>
</tbody>
</table>

NV126     NV126   110  On autosomes only

[The table continues with more entries.]

The autosomal NV126 probe was used as control for homogenization efficiency in a dot-blot assay. Data are from EICKBUSH, EICKBUSH and WERREN (1992).
profiles, \( F_3 \) deletion lines were established from chromosomes that were profiled in the \( F_1 \) generation, by taking a single \( F_2 \) male from each deletion line for further maintenance. Profiles were also established from these \( F_3 \) deletion lines. When \( F_3 \) profiles were different from the original \( F_1 \) (10/21 chromosomes, see Results), the \( F_3 \) profile was used.

A few deletion chromosomes (5 and 1 in the IR and CI method, respectively) had reduced DNA load on the filters, i.e., they hybridized weakly to every probe, even the autosomal \( NV126 \) control probe. Because no \( F_2 \) progeny were available from these chromosomes for additional profiling, their signal intensities were adjusted for poor homogenization.

Signal intensities of sample dots were scored as follows: 1 to 1/2 = "+" (present, no deletion); 1/4, 1/8 and 1/16 = "w" (weak, partial deletion) and <1/16 = "-" (absent, complete deletion). Thus, sample dots that had hybridization intensities equal to reference a "1/2" were (conservatively) scored as representing no deletions. In most cases, a background hybridization to \( psr79 \) was scored as "-", but may occasionally have been scored as "w" (e.g., if autosomal background hybridization was 1/16). Therefore, some complete deletions of \( psr79 \) may have been scored as partial.

**RESULTS**

**Effect of irradiation and incompatibility on PSR:** Deletions in the PSR chromosome were observed in males irradiated either in the pupal stage or as adults. Irradiated PSR males always produced all-male families (irradiated as pupae \( n = 21 \), irradiated as adults \( n = 71 \)). At low doses, family sizes of irradiated PSR males were not reduced compared with nonirradiated controls. In contrast, irradiated control (non-PSR) males had reduced family sizes, due to mortality of fertilized (diploid) eggs. For example, in the 3-Krad study, progeny sizes of nonirradiated control males were 23.0 ± 7.7 SD (\( n = 13 \)) versus 24.2 ± 5.9 SD (\( n = 19 \)) for irradiated PSR males (Mann-Whitney U-test; \( z = 0.424, P = 0.672 \)). Irradiated non-PSR males gave 11.5 ± 7.1 SD (\( n = 34 \)), which is a 50% reduction from the nonirradiated control (Mann-Whitney U-test; \( z = -3.795, P = 0.0001 \)). The increased mortality is most likely due to aneuploidy and dominant lethals from the irradiated sperm in control crosses. In contrast, irradiated PSR sperm did not cause increased mortality because the paternal chromosomes were eliminated by PSR action.

Even though a number of the irradiated PSR chromosomes had undergone deletions that made them nonfunctional in the \( F_1 \) cross, they were still functional in the parental male after irradiation, as evidenced by the fact that they ended up in male progeny. At 20 Krad, progeny sizes of irradiated PSR males were smaller than nonirradiated males (21.9 ± 10.6 SD, \( n = 29 \) vs. 37.5 ± 5.2 SD, \( n = 6 \); Mann-Whitney U-test; \( z = -5.088, P = 0.0002 \)). Family sizes of irradiated non-PSR males were very small and all-male (6.2 ± 3.1 SD, \( n = 10 \)). Thus, higher doses of irradiation resulted in some lethality of PSR fertilized eggs and complete lethality of wild-type fertilized eggs. The partial lethality in PSR fertilized eggs may be due to increasing harmful effects of irradiation on PSR expression, resulting in survival of paternal chromosomes and subsequent expression of dominant lethals, as seen in the irradiated controls. Alternatively, irradiation may affect other properties of the sperm that are essential for proper development of fertilized eggs.

The proportion of PSR chromosomes surviving the irradiation, measured as the proportion of \( F_1 \) males that were carriers of PSR, decreased with increasing dose from around 85% at 3 Krad (\( n = 517 \)) to 20% at 20 Krad (\( n = 160 \)). The fraction of surviving chromosomes that contained detectable deletions increased with increasing doses (i.e., 0.2% at 3 Krad and 3.1% at 20 Krad). A total of 88 PSR chromosomes with detectable deletions were obtained by irradiation. These chromosomes were detected because they partly or completely lacked one or more of the repeat types used in the screening procedure. Obviously, using our screening method, PSR chromosomes containing small deletions or deletions in regions for which we have no probes could have been overlooked.

A second method for generating deletions was cytoplasmic incompatibility (CI). In the CI crosses, \( F_1 \) progeny were also always all-male (haploid) due to the elimination of the paternal chromosomes in fertilized eggs. This was to be expected because both CI and PSR cause paternal chromosome elimination. PSR chromosomes generally survived incompatibility at low frequency of approximately 5%. Thus, it can be concluded that although PSR is immune to its own effects, it is not immune to effects of cytoplasmic incompatibility, which is caused by a symbiotic microorganism that presumably imprints the paternal chromosomes (Ryan and Saul 1968; Breuer and Weren 1990). In one experiment, the PSR chromosome was found in 51 (4.3%) of 1187 \( F_1 \) males. Among those, 20 (39%) contained detectable deletions. A total of 51 deletion chromosomes were obtained by cytoplasmic incompatibility.

**Types of PSR deletion chromosomes:** All deletion chromosomes present in \( F_1 \) males were categorized based upon the sex ratios they produced and which offspring sex inherited the chromosome (Table 2). Progeny sex ratios varied from female-biased (stand-
ard M1) to all-male. Within each lineage PSR was detected either (1) only in sons, (2) only in daughters, (3) in both or (4) in neither sex. Functional (F) PSR deletion chromosomes were found only in F2 sons, because they still destroyed the paternal chromosomes, thus, converting diploid eggs (females) into haploid (males). F chromosomes were found both among all-male families and in mixed (male and female) sex ratio families. In contrast, nonfunctional (NF) PSR deletion chromosomes were found in F2 daughters but not sons; because they no longer destroyed the paternal chromosomes and thus ended up in females (i.e., eggs fertilized by nonfunctional PSR chromosomes remain diploid and female).

A total of 40 deletion chromosomes were functional and 23 were nonfunctional. With one exception, all chromosomes examined remained functional or nonfunctional, respectively, over subsequent generations. In addition, with the exception of two crosses, no chromosomes were found to be present in both male and female F2 progeny. These exceptions will be discussed later. Sixty-four chromosomes were found to be transmitted to neither F2 sons nor daughters and are typified as "unknown functionality" (UF). Twelve additional CI-generated chromosomes were unclassified because F2 progeny were not screened for presence of PSR.

Transmission of PSR deletion chromosomes: Preliminary characterization indicated that transmission rates of deletion chromosomes were generally lower and varied more than standard (wild-type) PSR chromosomes. Wild-type PSR males transmit the chromosome to 94–100% of fertilized eggs (BEUKEBOOM and WERREN 1993). They occasionally produce daughters, which do not inherit PSR. Functional deletion chromosomes were transmitted at rates of 48–100% from fathers to sons. Transmission rates of nonfunctional deletion chromosomes were generally high (around 75%) through males and low (around 10%) through females. Thus, it can be concluded that deletions tended to increase the instability of these chromosomes. In addition, nonfunctional PSR chromosomes appear to have poor transmission through female (meiotic) gametogenesis. A more detailed analysis of transmission rates of deletion chromosomes will be presented elsewhere (L. BEUKEBOOM and J. WERREN, in preparation).

Profiles of PSR deletion chromosomes: Molecular profiles of all deletion chromosomes were established by hybridizing their homogenates to seven repetitive DNA probes and comparing their signal intensities with serially diluted homogenates from males carrying a standard (wild-type) PSR chromosome, as described in Methods. Profiles of deletion chromosomes in each of the four classes are shown in Table 3. As can be seen, IR and CI generated profiles were very similar. Results indicate that most repeat types can be entirely deleted and therefore are organized in blocks on the chromosome.

Figure 3 shows the deletion probability for each repeat type. In decreasing order, the deletion probabilities are: psr105 > psr10 > psr2 > psr79 > psr22 > psrl3 > pslr18. Psrl3 was only deleted in four (type UF) of 139 chromosomes, and in no chromosomes was pslr18 found to be completely deleted. Recall that pslr10 and pslr105, as well as psr13 and pslr18, partly cross-hybridize to each other even at high stringency, but that each hybridizes more intensely to itself than to the other. This is also apparent in the deletion profiles. For example, some chromosomes show strong hybridization to pslr18 and weak to pslr13, and others the opposite pattern.

Most deletions involved more than one repeat type. Repeat type pslr79 and pslr10&105 were sometimes deleted independently of the remaining repeats (Table 3). In contrast, repeat types psr2, pslr22 and pslr13&18 were never deleted by themselves. Figure 4 shows for each completely deleted repeat type the probability that any of the other repeat types were also deleted from the same chromosome. It can be seen that whenever psr2 is deleted, pslr10&105 are also deleted from the chromosome and whenever pslr22 is deleted both psr2 and pslr10&105 are also absent. Deletions of pslr79 often occur together with pslr10&105, psr2 and pslr22. Repeat types that are frequently deleted together may be adjacent on the chromosome. The pattern in deletions indicates a hierarchical order in repeat organization, i.e., pslr22 next to pslr2 and pslr10&105. Although pslr79 may also be part of this order, its independence is suggested by the finding that it can be deleted by itself (Table 3). Ten deletion chromosomes have been examined cytologically (K. REED, unpublished results). All show visible deletions, which are consistent with the apparent size based upon probing. For example, one chromosome (ID# N016) has only repeat type pslr13&18 left and has apparently lost both chromosome arms. It is visible as a "dot" compared with the standard submetacentric PSR chromosome.

Association of function with repeat type: Deletion probes can be used to determine if particular regions of the chromosome are associated with PSR action. Table 4 shows how often complete (―) or partial (w) deletion of a particular repeat type results in loss of function. Four repeat families (psr79, pslr2, psr10, pslr105) were sometimes completely deleted without loss of function, e.g., complete deletions of psr2, pslr10 and pslr105 occurred about equally on F and NF chromosomes. Partial deletions of all repeat types (except pslr105) are found on both F and NF chromosomes. Complete deletion of pslr79 showed a high probability of function loss (12 of 14), although it
TABLE 3
Profiles of PSR deletion chromosomes

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<tr>
<th>Functional deletion chromosomes (PSR repeat type)</th>
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</tr>
<tr>
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<td>- + + + + + +</td>
<td>1</td>
</tr>
<tr>
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</tr>
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</table>

Deletion chromosomes are classified according to Table 2. Profiles were determined by comparing hybridization intensities of sample dots with serially diluted reference dots from wild-type PSR chromosomes in a dot-blot assay (see Figure 2). Intensities were transformed as follows: 1 and $\frac{1}{2}$ = + (present or no deletions), 1/4, 1/8 and $\frac{1}{16}$ = w (weak or partial deletions), $<\frac{1}{16}$ and 0 = - (absent or complete deletions). Psrl3 and psrl8 cross-hybridize, as do psrl0 and psrl05. Profiles with weak hybridization to every PSR repeat type are listed below the dotted line. The total number of deletion chromosomes with each profile is given. The ones created by CI are indicated between brackets, all others are IR generated.
Deletion Analysis of PSR

**FIGURE 3.** Frequency of deletion of each type of PSR repeat. Four types of deletion chromosomes are distinguished (F = functional, NF = nonfunctional, UF = unknown-functionality and NC = nonclassified). Data for all categories combined are also shown. Solid bars represent complete deletions (“**” = no hybridization signal) and open bars partial deletions (“w” = weak hybridization signal).

could be deleted with function retention. *Psr22* is noteworthy because all six complete deletions of the repeat resulted in loss of function. Thus, *psr22* may be linked with a functional domain of *PSR*. In all six cases where *psr22* is deleted, *psr2*, *psr10* and *psr105* are also deleted (see NF deletion chromosome profiles, Table 3). Therefore, deletions that have removed *psr22* tend to be large, and may have a higher probability of removing the functional domain(s) of

**FIGURE 4.** Conditional frequencies of complete deletions of each PSR repeat type. Above each graph it is indicated how many times the repeat is completely deleted (n). Shown is the probability of complete deletion for any given repeat type (indicated above each graph), given that the repeat type indicated on the horizontal axis is completely deleted (solid bars). The overall probability that the repeat type is deleted is shown by the open bar. For example, if *psr22* is deleted, *psr2*, *psr10* and *psr105* are nearly always deleted, but the reverse is not true.
different chromosome variants (two cases, discussed
odds. Ten chromosomes showed differences from the
observation that single F1 males (the generation im-
mediately following the irradiation) can carry two
deletion chromosomes. To avoid this complica-
tion, we established F3 deletion lines from 21 of
lines that had been profiled in the F1 generation.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Deletion</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>F599</td>
<td>None</td>
<td>Functional</td>
</tr>
<tr>
<td>F202</td>
<td>Partial</td>
<td>Nonfunctional</td>
</tr>
<tr>
<td>F301</td>
<td>Complete</td>
<td>Nonfunctional</td>
</tr>
</tbody>
</table>

Unusual deletion chromosomes: Because of the
observation that single F1 males (the generation im-
mediately following the irradiation) can carry two
different chromosome variants (two cases, discussed
later), some profiles could represent a melding of two
different deletion chromosomes. To avoid this com-
plication, we established F3 deletion lines from 21 of
40 lines that had been profiled in the F1 generation.
This could only be done for deletion chromosomes that were transmitted (F and NF) and maintained.
These F3 lines were then profiled by standard meth-
ods. Ten chromosomes showed differences from the
F1 male profile. Eight of these did not show a change
in the actual profile, but rather an increase in intensity
of hybridization to every repeat type in some individ-
uals. This interindividual variation in hybridization
intensity to every repeat type persisted in subsequent
generations (6 chromosomes tested). The result is
consistent with the view that the original male was
mosaic for PSR bearing and nonbearing cells and that
the chromosome subsequently remained mitotically
unstable.

Three IR induced deletion chromosomes (ID# E288, I002 and F599) showed changes in profiles (Table 5). These are discussed below.

Deletion chromosome #E288: E288 (NF chromosome) showed profile changes between F1 and F3. It hybrid-
ized to each probe in the F1, but lacked repeat types
psr2 and psr10&105 in the F3 profile. The most likely explanation is that the original F1 male carried two
different deletion chromosomes; (1) a UF chromo-
some that contained repeat types psr2 and psr10&105, but was not transmitted and (2) a NF chromosome
that lacked psr2 and psr10&105, but was transmitted
to F2 daughters. Alternatively, the F3 chromosome
may have been generated in the F1 male or F2 female
deliction.

Deletion chromosome I002: Two deletion chromo-
somes (ID# I002 and F599) showed an exceptional
transmission pattern. These were originally found to
be transmitted to both F2 sons and daughters, sug-
gesting that at times they were both functional and
nonfunctional. We investigated these further in sub-
sequent crosses. The F1 male line (#I002) appeared
to have two distinct phenotypes. F2 carrier males trans-
mitt the chromosome only to their F3 sons and not
to their F3 daughters, indicating it to be a functional
chromosome. However, F3 males from the virgin F2
carrier females (who received the nonfunctional "phase" from the F1 male) transmitted the chromo-
some to only their F4 daughters and not their F4 sons.
These F3 males, therefore, had inherited a NF chro-
mosome from their mother. Both types were subse-
quetly found to have different deletion profiles (Table 5). The significance of these profile differences
is unclear.

Deletion chromosome F599: The other (#F599) chro-
mosome had a more complicated inheritance pattern
(Figure 5). It showed a change from NF to F in some
lineages, but not others. Both F2 males and females
probed positive for the chromosome. F2 male carriers
produced all-male offspring and transmitted the chro-
mosome to their F3 sons. F3 sons, in turn, transmitted
it to their F4 sons only. They therefore had inherited
a fully functional chromosome. In contrast, F3 sons
from F2 virgin female carriers transmitted the chro-
mosome to both some of their F4 sons (2 of 33) and F4
daughters (76 of 167). Thus, although F3 males had
inherited an apparently nonfunctional chromosome
from their mother, they transmitted a chromosome
that was sometimes functional (to 2 of their F4 sons)
and sometimes nonfunctional (to 45.5% of their

**Table 4**

<table>
<thead>
<tr>
<th>PSR repeat type</th>
<th>79</th>
<th>18</th>
<th>15</th>
<th>22</th>
<th>2</th>
<th>10</th>
<th>105</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>15</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>w</td>
<td>14</td>
<td>6</td>
<td>22</td>
<td>42</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4</td>
<td>15</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>18</td>
<td>13</td>
<td>34</td>
<td>25</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

For example, 12 of 14 chromosomes that had psr79 completely deleted were nonfunctional.

**Table 5**

<table>
<thead>
<tr>
<th>Molecular profiles of three F1 male lineages that contained more than one deletion chromosome (see text for explanation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID#</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>E288</td>
</tr>
<tr>
<td>E288a</td>
</tr>
<tr>
<td>I002</td>
</tr>
<tr>
<td>I002a</td>
</tr>
<tr>
<td>I002b</td>
</tr>
<tr>
<td>F599</td>
</tr>
<tr>
<td>F599a</td>
</tr>
<tr>
<td>F599b</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Deletion Analysis of PSR

Nevertheless, F599 did spawn functional chromosomes from nonfunctional variants, although these were not stably transmitted. The significance of deletion chromosomes that change profile or function over subsequent generations needs to be further investigated.

DISCUSSION

The deletion analysis confirms that individual repeat types are organized in blocks, rather than widely interspersed on the chromosome. Further evidence for this comes from analysis of lambda clones of repetitive DNA from PSR. These clones (with 10–20 kb inserts) typically contain large, uniform blocks of single repeat types (Eickbush, Eickbush and Werren 1992). Repeat types psr13 and psr18, which are never completely deleted may be located at or close to the centromere. Frequently deleted repeat types, such as psr2, psr10 and psr105 are likely to be more distal on the chromosome. This reasoning is based on the notion that terminal deletions (one-break) are more likely to occur than interstitial deletions (two-breaks plus an annealing). An alternative interpretation is that the frequency of deletion reflects the size of a repeat type rather than the location. For example, repeat types that are restricted to a small chromosomal region are more likely to be deleted than ones stretching over larger regions. Profiles also suggest that psr22, psr2, psr10 and psr105 occur near each other, with psr22 most proximal to the centromere and psr10 and psr105 most distal. Repeat type psr79 is sometimes completely deleted independent of all other repeats (see Table 3), and could be located most distally on PSR. Verification of these interpretations await in situ hybridizations using the repeats.

Frequent occurrence of terminal deletions may seem in contrast with data from Drosophila, which indicate that most deletions are interstitial (Ashburner 1989). However, results from Drosophila are based on deletions in vital chromosomes. Therefore, large terminal deletions will frequently be lethal and thus not recovered. In contrast, because PSR is a nonvital B chromosome, large terminal deletions will not be lethal and may be much more common.

PSR chromosomes with terminal deletions are likely not to have telomeres, which may explain why they often become unstable somatically. Biersmann and Mason (1988) have generated X chromosomes in Drosophila that lack functional telomeres and showed that their breakpoints recede at a rate of about 75 bp per generation. Broken chromosome ends in yeast can heal to produce stable terminal deficiencies (Haber and Thorburn 1984; Haber et al. 1984). Chromosome fragments can also be maintained through several cell cycles, depending on the tissue and developmental stage (McClintock 1941a; Hughes-
Such chromosomes are sometimes capped by telomeres (reviewed by Blackburn and Szostak 1984). It is therefore possible that the instability of some deletion chromosomes (e.g., UF chromosomes) and the generation of multiple deletion chromosomes from single F1 male lines (#1002 and #F599) could be caused by the loss of telomeres and resulting breakage-fusion cycle. As McClintock (1941b, 1942) showed, such a cycle leads to chromosomes with altered sizes and chromatin constitution that sometimes subsequently become stable.

One characteristic of wild-type PSR is its nearly complete transmission to sperm (Beukeboom and Werren 1993). This is due to the fact that spermatogenesis is mitotic in haploid males. In contrast, PSR deletion chromosomes varied greatly in transmission stability. UF chromosomes were completely unstable, whereas NF and most F chromosomes had reduced transmission relative to wild-type chromosomes. A detailed analysis of transmission rates and mitotic stability of deletion chromosomes is presented in L. Beukeboom and J. Werren (in preparation) and Beukeboom, Reed and Werren (1992). Certain PSR repeats appear to be essential for stability of the chromosome. Cytogenetic evidence suggests that psr13&18 are at or close to the centromere. For example, the NF chromosome (#N016) has all repeats except for psr13&18 deleted and appears as a "dot" compared with the standard submetacentric PSR chromosome (K. Reed, unpublished results). This is consistent with the observation that all deletion chromosomes contain psr13&18.

It is interesting that two very different methods (irradiation and cytoplasmic incompatibility) give rise to such similar deletion profiles. Based on deletion chromosome profiles, CI caused slightly larger (more complete) deletions, whereas IR more frequently resulted in chromosomes with only a single repeat type being partially deleted.

Profiles that showed weak hybridization to every PSR probe require explanation. Do such profiles represent chromosomes containing deletions in every repeat type? Upon further investigation (Beukeboom, Reed and Werren 1992) we found that such profiles are due to mosaicism: the presence of the deletion chromosome in some tissues of the wasp, but not in others. Absence of the deletion chromosome in some tissues leads to weak hybridization signals to each probe. Individual carriers were found to differ in the degree of mitotic instability of the chromosome. This was also apparent upon establishing profiles from F3 deletion chromosome lines. It was found that in many individuals hybridization to each repeat type probe increased relative to the F1 profile. The study further indicated that incomplete transmission of deletion chromosomes is due to males having mosaic testes, resulting in two types of sperm: carrier and noncarrier of the chromosome.

PSR function can be lost by deletions in the chromosome. A consistent difference between F and NF chromosomes was that all complete deletions of psr22 were associated with function loss. This suggests that a functional domain of PSR may map close to psr22. However, the results are also consistent with function, depending on actual abundance of repeats on the chromosome. This is because all deletion chromosomes that had psr22 deleted also had lost psr2, psr10 and psr105. From F chromosome profiles, it is clear that PSR action does not require the presence of repeat types psr10, psr105, psr2 and psr79. Psr13 and psr18 can be partly deleted without affecting PSR function, but because these repeat types are never completely deleted in F chromosomes, they can not be excluded from linkage to PSR function. NF chromosomes can have psr13, psr18, psr79, psr22 or psr2 completely present. However, partial deletions in these repeat types could have remained undetected due to the coarse screening method used.

We do not know the exact timing of PSR action. Modification of the paternal chromosomes could occur during spermatogenesis or in the short time period between fertilization and the first cleavage division of the zygote. We observed that all irradiated males still produced all-male offspring and these offspring inherited PSR, even though some of them apparently received a nonfunctional PSR chromosome. Moreover, some of these males were irradiated at the 9-day-old pupal stage, which coincides with early spermatogenesis (Hogge and King 1975). This suggests that either (i) the autosomes have already been modified by PSR in early spermatogenesis, or (2) fragmentation of the chromosome in sperm does not interfere with its functioning in the early fertilized egg. In the latter case, resulting males may then transmit a nonfunctional deletion chromosome to their F2 daughters.

Identifying the regions of the PSR chromosome responsible for PSR action is an essential step toward understanding the genetic mechanism of this element. Eickbush, Eickbush and Werren (1992) proposed two alternate mechanisms of PSR action. First, the PSR chromosome may contain one or a few unique genes that code for a product (i.e., DNA binding protein or methylase) that prevents proper processing of paternal chromosomes. Alternatively, sequences on PSR may act as a binding site ("sink") for a product required for paternal chromosome condensation and/or replication.

A possibility is that the repeats themselves are the functional domains of PSR, i.e., by binding away an essential protein for proper processing of paternal chromosomes. A line of evidence supporting this hy-
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The hypothesis is that all PSR specific repeats contain two highly conserved palindromic segments (Eickbush, Eickbush and Werren 1992). Palindromes can act as binding sites for proteins (Lee et al. 1987; Davidson and Saintgirons 1989; Rissee et al. 1989; Halazonetis and Kandil 1991). Under this hypothesis, we would not expect to find a single chromosomal region linked with PSR action, but rather function being determined by dosage of PSR specific repeats containing the palindromic sequences. The F599 chromosome that switched from nonfunctional to functional is of interest in this respect. Based upon its profile, F599 is the largest of the NF chromosomes (L. Beukeboom, unpublished results). Therefore, it may be close to the threshold number of repeats necessary for function. A determination of whether function maps to a specific region of PSR or to overall abundance of PSR specific repeats awaits finer scale mapping of the chromosome.

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LITERATURE CITED


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