Copyright © 1993 by the Genetics Society of America

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

Leo W. Beukeboom[1] and John H. Werren

Department of Biology, University of Rochester, Rochester, New York 14627

Manuscript received May 3, 1992
Accepted for publication November 25, 1992

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 *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 of 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 PEITZ 1985; SEITZ and BENNET 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 EICKBUSH 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

[1] Current address: Arbeitsgruppe Michiels, Max-Planck-Institut für Verhaltensphysiologie, D-8130 Seewiesen (Post Starnberg), Germany.

DNA so far described (WERREN, NUR and WU 1988; GODFRAY and HARVEY 1989; SHAW and HEWITT 1990).

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). Analysis of clones and genomic Southerns suggested that the majority of sequences comprising the *psr2, psr18* and *psr22* families are present on the *PSR* chromosome in long tandem arrays (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 to: (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 (WHITING 1967). It can easily be maintained in the laboratory (see WHITING (1967) WERREN (1991) and BEUKEBOOM and WERREN (1992) for details on biology and culturing methods). Generation time is 14 days at 25°. 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). F_1 males were crossed to virgin females from the MI strain. After mating, F_1 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 F_2 generation by whether the chromosome was transmitted to male progeny (indicating *PSR* action) or female progeny (loss of action) (Figure 1).

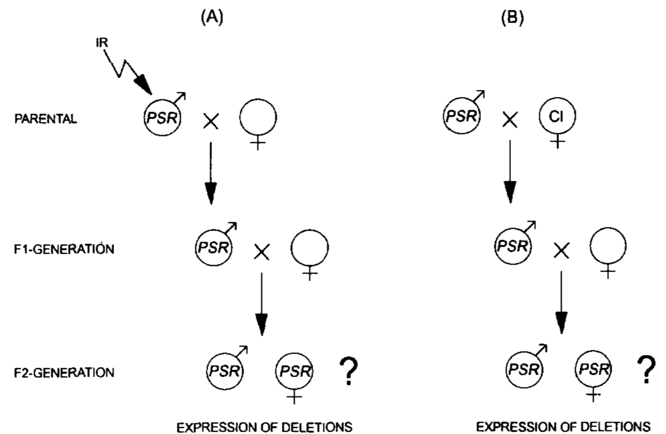

FIGURE 1.—Mating scheme for obtaining *PSR* deletion chromosomes by irradiation and cytoplasmic incompatibility. Parental males are (A) irradiated and mated to standard females (–), or (B) mated to incompatible females (CI). F_1 families are always all-male and some males inherit a *PSR* chromosome with deletions. Such males are identified in a dot-blot assay after mating to standard females. Deletion chromosomes are classified according to progeny sex ratios and transmission to sons or daughters.

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 ti277 females resulted in all-male families and survival of the *PSR* chromosome at low frequency (±5%). 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 F_1 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 F_1 to F_2 was done by probing 5 pooled F_2 progeny of each sex.

Dot-blot assay: The *PSR* chromosome was detected by hybridizing total wasp DNA to *PSR* specific probes. Radio-

TABLE 1

DNA repeat types used as probes to screen for deletions in the *PSR* chromosome

Repeat family	Repeat type	Repeat length (bp)	Miscellaneous
psr2	*psr2*	171	Specific to *PSR*
psr18			Specific to *PSR*
	psr10	207	Cross-hybridize even
	psr105	214	at high stringency
	psr13	212	Cross-hybridize even
	psr18	213	at high stringency
psr22	*psr22*	183	Specific to *PSR*
psr79	*psr79*	94	Present on *PSR*, and on autosomes at lower abundance
NV126	*NV126*	110	On autosomes only

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

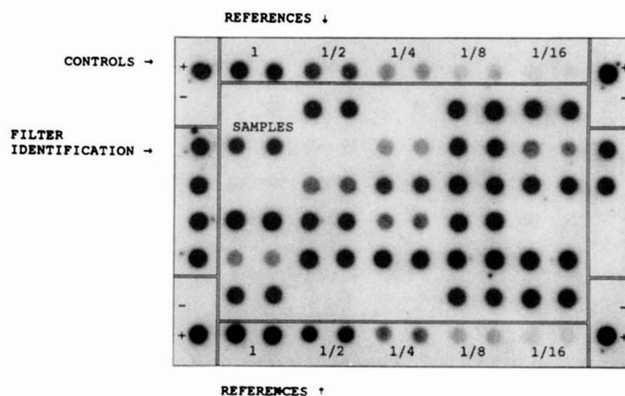

FIGURE 2.—Example of a filter used in the profile screening of *PSR* deletion chromosomes. Two boxes labeled "REFERENCE" contain duplicate dots of serially diluted homogenate from one male carrying a standard (wild-type) *PSR* chromosome. The box labeled "SAMPLES" contains duplicate dots of individual males carrying *PSR* deletion chromosomes. The "CONTROL" boxes at each corner contain undiluted dots of a carrier (+) and a noncarrier (−) of a standard *PSR* chromosome and serve as verification for the *PSR* specificity of the assay. The purpose of dots in the box "FILTER IDENTIFICATION" is to individually label filters. One of eight copies of each filter is hybridized to a single repeat probe (listed in Table 1). Within each filter, hybridization intensities of sample dots from *PSR* deletion chromosomes are compared with the reference dots. Shown is a filter hybridized to the *psr2* probe.

active probes were prepared from lambda clones containing seven different repetitive DNAs specific to *PSR* (NUR *et al.* 1988; EICKBUSH, EICKBUSH and WERREN 1992). Wasps were ground in 100 μl homogenization buffer (0.2 M NaCl, 0.2 M Tris, 0.02 M EDTA, 2% SDS, pH7). The solution was mixed with 10 μl [2.5 mg/ml] proteinase K and incubated at 50° for 1 hr. The DNA was denatured with 1/5 volume NaOH and the solution neutralized with 1/5 volume Tris and 1/5 volume HCl. One microliter of the resulting solution was spotted onto nitrocellulose filter, which was then dried and baked at 80° in a vacuum oven or UV cross-linked for 30 sec at 120 mJ. Filters were prehybridized for 4 hr and hybridized overnight with the *PSR* probe at 65° (normal stringency). Prehybridization and hybridization solutions were 2× SSC, 5× Denhardt's, 1% sodium pyrophosphate, 25 mM sodium phosphate, [250 mg/ml] denatured ctDNA and 1% SDS in distilled water. *PSR* probes were labeled using the random priming DNA labeling method (Amersham kit) with ^{32}P labeled ATP. After four stringency washes with decreasing concentrations of salt (4×, 2×, 1×, 0.1× SSC + 1% SDS) filters were dried and exposed to autoradiographic film for 1–4 days in −80°. *PSR* carrying males were easily scored by this method, because normal males give no signal.

Profiling deletions: Deletions in the *PSR* chromosome were detected by hybridizing DNA of individual carrier males to radioactive labeled probes from lambda clones containing *PSR*-specific repeats. Each deletion chromosome was characterized for presence of one of seven *PSR* repeat types (Table 1). Individual repeat lengths varied from 94 bp (*psr79*) to 214 bp (*psr105*). Probes ranged in size from two to a few dozen repeat units. All repeats are specific to *PSR*, except *psr79*, which is also present on the autosomes, but enriched on *PSR*. No cross-hybridization occurs at normal stringency between repeat families. Within the *psr18* family, repeat types *psr10* and *psr105* do not cross-hybridize with *psr13* and *psr18* at high hybridization stringency (75° and 4×SSC) and could be used as separate probes. However, *psr10* and *psr105*, as well as *psr13* and *psr18*, still cross-hybridize at high stringency although each hybridizes stronger with itself than with the other. They will be referred to as *psr10&105* and *psr13&18*.

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.

DNA solutions were prepared as described for the dot-blot assay and dotted in duplicate onto eight replicate filters. 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 1/16×). 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 autoradiographic film had minimal effect on the analysis. Nevertheless, in the CI method films were preflashed to work in the linear 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.

Molecular profiles of deletion chromosomes were first established from individual F_1 males. Subsequently, individual F_2 progeny were used for profiling. To confirm F_1

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 obtained 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 Krads, 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 = −3.088, P = 0.002). 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.

TABLE 2

Types of *PSR* deletion chromosomes defined by their progeny sex ratio and presence or loss of *PSR* function

Deletion chromosome	Functionality	Transmission	Progeny sex-ratio	Carrier sex
Type F	Functional	Complete or incomplete	All-male or high-male	Males
Type NF	Nonfunctional	Incomplete	Female-biased	Females
Type UF	Unknown	No	Various	None

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; BREEUWER and WERREN 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 MI) 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 F_2 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 F_2 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 F_2 progeny. These exceptions will be discussed later. Sixty-four chromosomes were found to be transmitted to neither F_2 sons nor daughters and are typified as "unknown functionality" (UF). Twelve additional CI-generated chromosomes were unclassified because F_2 progenies 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* > *psr13* > *psr18*. *Psr13* was only deleted in four (type UF) of 139 chromosomes, and in no chromosomes was *psr18* found to be completely deleted. Recall that *psr10* and *psr105*, as well as *psr13* and *psr18*, 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 psr18 and weak to psr13, and others the opposite pattern.

Most deletions involved more than one repeat type. Repeat type *psr79* and *psr10&105* were sometimes deleted independently of the remaining repeats (Table 3). In contrast, repeat types *psr2*, *psr22* and *psr13&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, *psr10&105* are also deleted from the chromosome and whenever *psr22* is deleted both *psr2* and *psr10&105* are also absent. Deletions of *psr79* often occur together with *psr10&105*, *psr2* and *psr22*. 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.*, *psr22* next to *psr2* and *psr10&105*. Although *psr79* 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). These all show visible deletions, which are consistent with the apparent size based upon probing. For example, one chromosome (ID# *N016*) has only repeat type *psr13&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 profiles 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*, *psr2*, *psr10*, *psr105*)were sometimes completely deleted without loss of function, *e.g.*, complete deletions of *psr2*, *psr10* and *psr105* occurred about equally on F and NF chromosomes. Partial deletions of all repeat types (except *psr105*) are found on both F and NF chromosomes. Complete deletion of *psr79* showed a high probability of function loss (12 of 14), although it

TABLE 3

Profiles of *PSR* deletion chromosomes

Functional deletion chromosomes (*PSR* repeat type)

79	18	13	22	2	10	105	Number
+	+	+	+	+	w	w	1
+	+	+	+	w	+	+	4
+	+	+	w	+	+	+	3
+	+	w	+	+	+	w	1
+	+	w	+	w	+	+	1
w	+	+	+	+	+	+	7
−	+	+	+	+	+	+	1
−	+	+	+	w	−	−	1
+	+	+	+	+	−	−	2 (1)
+	+	+	w	+	−	−	2
+	+	+	w	w	−	−	2
+	w	w	+	w	−	−	1
+	w	+	w	w	−	−	1
+	+	+	+	−	−	−	2 (1)
+	+	+	w	−	−	−	5 (3)
+	w	w	w	−	−	−	2
w	w	w	w	w	w	w	2
w	w	w	w	w	−	−	2
							40 (5)

Nonfunctional deletion chromosomes (*PSR* repeat type)

79	18	13	22	2	10	105	Number
w	+	+	w	w	w	−	1
w	+	+	w	+	−	−	1 (1)
w	+	+	w	w	−	−	1
w	w	+	w	w	−	−	2
−	+	+	w	w	−	−	1
−	w	+	w	w	−	−	2
+	+	+	w	−	−	−	1 (1)
−	+	+	+	−	−	−	2 (2)
−	+	+	w	−	−	−	1
w	+	+	w	−	−	−	1
w	w	+	w	−	−	−	2
w	+	+	−	−	−	−	1 (1)
−	+	+	−	−	−	−	3 (1)
w	w	w	w	w	−	−	1
−	w	w	w	w	−	−	1
−	w	w	−	−	−	−	2 (1)
							23 (7)

Unknown-functionality deletion chromosomes (*PSR* repeat type)

79	18	13	22	2	10	105	Number
+	+	+	+	w	+	+	2 (1)
+	+	+	w	+	+	+	1
w	+	+	+	+	+	+	1
+	+	+	+	+	w	w	1 (1)
w	+	+	w	+	+	+	1

Unknown-functionality deletion chromosomes (*PSR* repeat type)

79	18	13	22	2	10	105	Total
w	+	w	+	+	+	+	1
+	+	w	+	+	w	w	1
+	w	w	w	w	w	w	1
−	w	w	w	+	+	+	1
−	+	+	w	+	w	w	1 (1)
−	+	+	w	+	−	−	2
+	+	+	+	+	−	−	1
+	+	+	+	w	−	−	1 (1)
+	+	+	w	+	−	−	3 (1)
w	+	+	+	+	−	−	1
w	+	+	w	+	−	−	1
w	+	+	w	w	−	−	1
w	w	+	w	w	−	−	1
w	w	+	w	+	−	−	1
+	w	w	+	+	−	−	1
+	w	w	+	w	−	−	1
+	w	w	w	−	−	−	1
w	w	w	+	−	−	−	1
−	w	w	+	−	w	w	1 (1)
+	w	w	−	−	−	−	1 (1)
−	w	+	−	−	w	w	1 (1)
−	+	+	−	−	−	−	1 (1)
w	w	w	w	w	w	w	2
−	w	w	w	w	w	w	1 (1)
w	w	w	w	w	−	−	3 (2)
w	w	−	w	w	−	−	1 (1)
−	w	w	w	w	−	−	3
w	w	w	w	−	−	−	5
−	w	w	w	−	−	−	1
−	w	−	w	−	−	−	1 (1)
w	w	w	−	−	−	−	2
−	w	w	−	−	−	−	12 (11)
−	w	−	−	−	−	−	2 (2)
							64 (27)

Nonclassified deletion chromosomes (*PSR* repeat type)

79	18	13	22	2	10	105	Total
+	+	+	+	+	−	−	1 (1)
+	+	+	+	w	−	−	2 (2)
+	+	+	+	−	−	−	1 (1)
+	w	+	w	−	−	−	1 (1)
+	+	+	−	−	−	−	1 (1)
+	w	w	−	−	−	−	1 (1)
w	w	w	w	w	w	w	1 (1)
w	w	w	w	w	w	−	1 (1)
w	w	w	w	w	−	−	1 (1)
w	w	w	−	−	−	−	1 (1)
−	w	w	−	−	−	−	1 (1)
							12 (12)

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 ½ = "+" (present or no deletions), ¼, ⅛ and 1/16 = "w" (weak or partial deletions), <1/16 and 0 = "−" (absent or complete deletions). *Psr13* and *psr18* cross-hybridize, as do *psr10* and *psr105*. 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 backets, all others are IR generated.

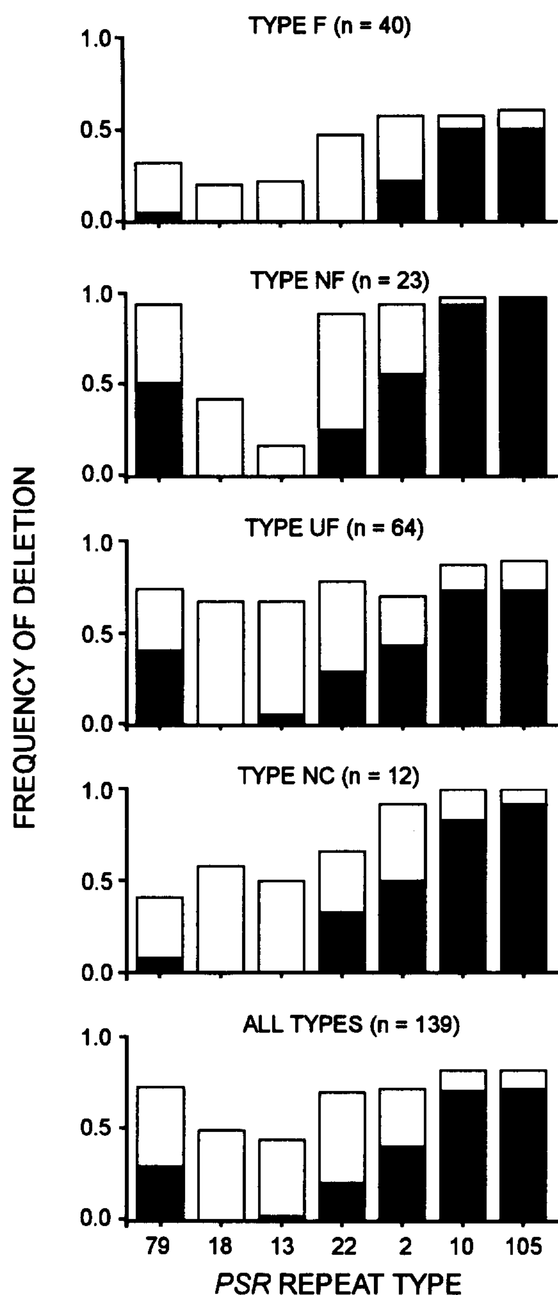

FIGURE 3.—Frequency of deletion of each type of *PSR* repeat. Four types of deletion chromosomes are distinguished (F = functional, NF = nonfunctional, UF = unknown-functionality and NC = nonclassified). Data for all categories combined are also shown. Solid bars represent complete deletions ("–" = no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

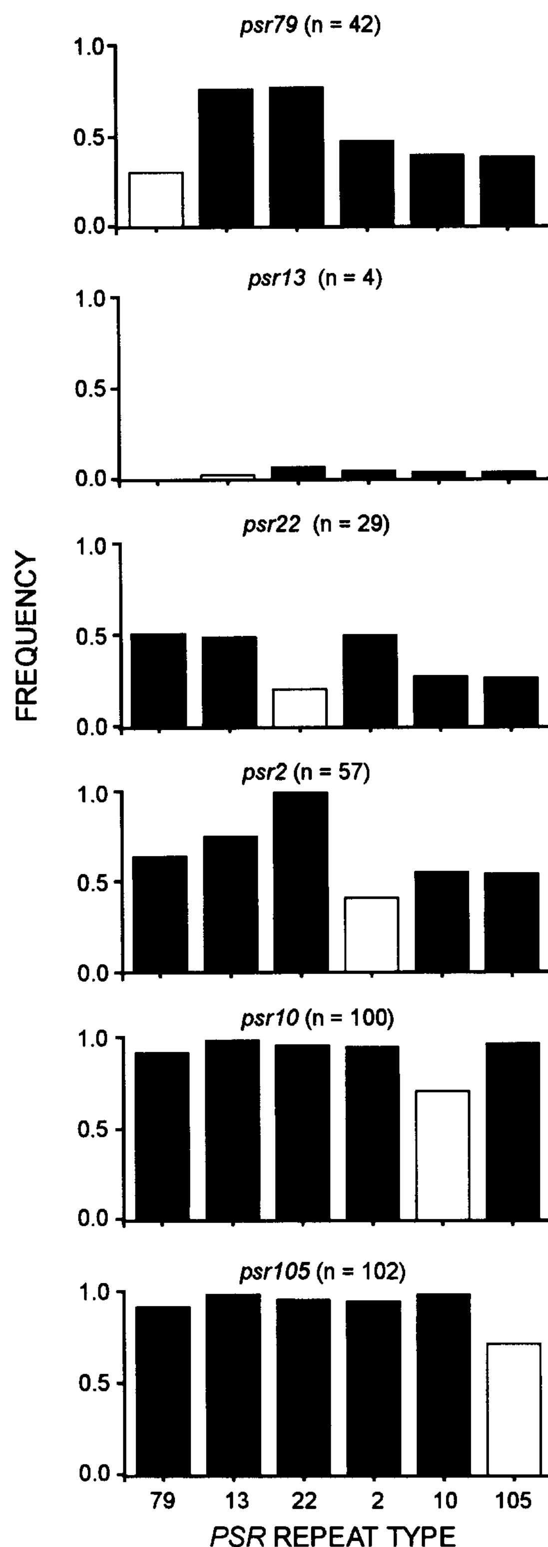

FIGURE 4.—Conditional frequencies of complete deletions of each *PSR* repeat type. Above each graph it is indicated how many times the repeat is completely deleted (n). Shown is the probability of complete deletion for any given repeat type (indicated above each graph), given that the repeat type indicated 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 in not true.

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

TABLE 4

Frequency of loss of *PSR* function associated with complete (−) and partial (w) deletion of each repeat type, relative to the number of occurrences

	PSR repeat type						
	79	18	13	22	2	10	105
−	$\frac{12}{14}$	0	0	$\frac{6}{6}$	$\frac{13}{22}$	$\frac{22}{42}$	$\frac{23}{43}$
w	$\frac{10}{21}$	$\frac{10}{18}$	$\frac{4}{13}$	$\frac{15}{34}$	$\frac{9}{23}$	$\frac{1}{4}$	$\frac{0}{4}$

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

PSR. The results are also consistent with function depending on actual abundance of repeats on the chromosome. Interestingly, some chromosomes show weak (or no) hybridization to every probe. These are listed in Table 3 below the ones that have at least one complete repeat type. They make up 33.1% ($n = 46$) of all profiles and were created through both IR and CI. The majority of such chromosomes are UF chromosomes (71.7%, $n = 33$). These chromosomes were probably mitotically unstable and therefore often lost prior to transmission to F_2 progeny. This would explain the low signal intensity for all repeat types.

Unusual deletion chromosomes: Because of the observation that single F_1 males (the generation immediately 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 complication, we established F_3 deletion lines from 21 of 40 lines that had been profiled in the F_1 generation. This could only be done for deletion chromosomes that were transmitted (F and NF) and maintained. These F_3 lines were then profiled by standard methods. Ten chromosomes showed differences from the F_1 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 individuals. 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 F_1 and F_3. It hybridized to each probe in the F_1, but lacked repeat types *psr2* and *psr10&105* in the F_3 profile. The most likely explanation is that the original F_1 male carried two different deletion chromosomes; (1) a UF chromosome 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 F_2 daughters. Alternatively, the F_3 chromosome may have been generated in the F_1 male or F_2 female by deletion.

TABLE 5

Molecular profiles of three F_1 male lineages that contained more than one deletion chromosome (see text for explanation)

		PSR repeat type						
ID#	Individual	79	18	13	22	2	10	105
E288	F1 male	w	+	+	+	+	w	w
E288a	F3 male	w	w	+	w	−	−	−
I002	F1 male	Not profiled						
I002a	F2 male	−	+	+	+	w	−	−
I002b	F2 female	−	w	w	w	w	−	−
F599	F1 male	+	+	+	w	w	+	+
F599a	F2 male	+	+	+	+	+	+	+
F599b	F2 female	w	w	w	w	w	−	−
	"Reverted" F4 male	w	w	w	w	w	−	−
	"Nonreverted" F4 male	w	w	w	w	w	−	−

Deletion chromosome I002: Two deletion chromosomes (ID# *I002* and *F599*) showed an exceptional transmission pattern. These were originally found to be transmitted to both F_2 sons and daughters, suggesting that at times they were both functional and nonfunctional. We investigated these further in subsequent crosses. The F_1 male line (*#I002*) appeared to have two distinct phenotypes. F_2 carrier males transmitted the chromosome only to their F_3 sons and not to their F_3 daughters, indicating it to be a functional chromosome. However, F_3 males from the virgin F_2 carrier females (who received the nonfunctional "phase" from the F_1 male) transmitted the chromosome to only their F_4 daughters and not their F_4 sons. These F_3 males, therefore, had inherited a NF chromosome from their mother. Both types were subsequently found to have different deletion profiles (Table 5). The significance of these profile differences is unclear.

Deletion chromosome F599: The other (*#F599*) chromosome had a more complicated inheritance pattern (Figure 5). It showed a change from NF to F in some lineages, but not others. Both F_2 males and females probed positive for the chromosome. F_2 male carriers produced all-male offspring and transmitted the chromosome to their F_3 sons. F_3 sons, in turn, transmitted it to their F_4 sons only. They therefore had inherited a fully functional chromosome. In contrast, F_3 sons from F_2 virgin female carriers transmitted the chromosome to *both* some of their F_4 sons (2 of 33) and F_4 daughters (76 of 167). Thus, although F_3 males had inherited an apparently nonfunctional chromosome from their mother, they transmitted a chromosome that was sometimes functional (to 2 of their F_4 sons) and sometimes nonfunctional (to 45.5% of their

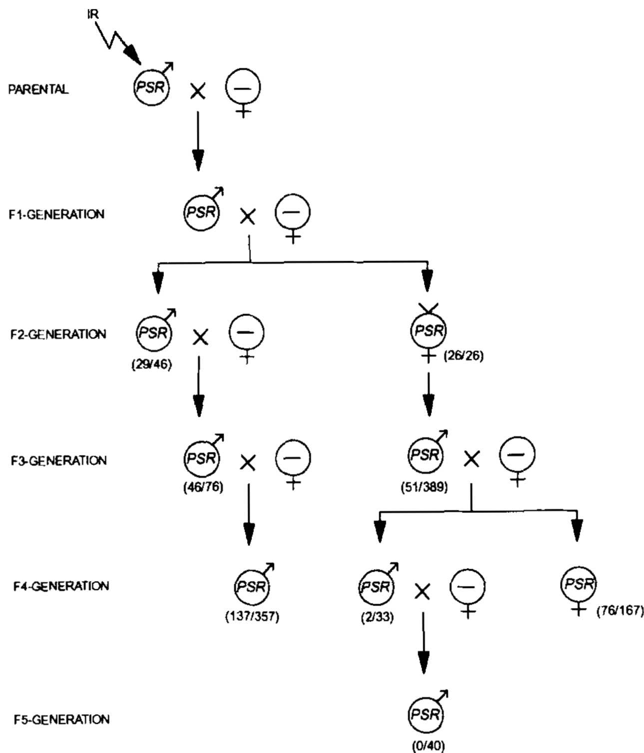

FIGURE 5.—Inheritance pattern of deletion chromosome *F599* that was originally transmitted to both F_2 sons and daughters. F_2 sons produced all-male offspring and transmitted the chromosome to their F_3 sons, which in turn transmitted it only to their F_4 sons. Therefore, F_2 sons had inherited a F chromosome. Virgin F_2 carrier females transmitted the chromosome to some of their F_3 sons. These F_3 sons were mated to standard (−) females and transmitted the chromosome to both some of their F_4 sons and F_4 daughters. Thus, although F_3 sons had inherited the chromosome in a "nonfunctional state" (because it came from their mother), they transmitted it sometimes as functional (2 of 33) and other times as nonfunctional (76 of 167). Both their F_4 sons did not transmit the chromosome further. F_5 sons from virgin F_4 carrier females transmitted the chromosome only to their F_6 daughters and not their F_6 sons (not shown). Thus, this chromosome switched from nonfunctional to functional. Numbers in brackets indicate how many of the tested offspring were carriers of the chromosome.

daughters). Both F_4 carrier males from the "revertant" chromosome produced all-male offspring. However, they did not transmit the chromosome to any of their F_5 sons (0 of 40). F_5 sons from virgin F_4 carrier females of that same lineage apparently retained a NF chromosome, since it was only transmitted to their F_6 daughters.

It is interesting to determine whether chromosome profiles differed with the different phenotypes from this deletion line. These are shown in Table 5. The original profiles differed in F_2 males *versus* F_2 females. However, we were unable to detect a subsequent change in profile associated with the switch of the NF (female) lineage to functionality. Profiles from the two "revertant" F_4 males did not differ from their "nonrevertant" F_4 brothers. Comparison of these profiles are complicated by the occurrence of mosaicism (*i.e.*, none of the repeat types showed full hybridization). 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 became unstable somatically. BIESSMANN 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-

SCHRADER and RIS 1941; BROWN 1960). 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 F_1 male lines (*#I002* 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 F_3 deletion chromosome lines. It was found that in many individuals hybridization to each repeat type probe increased relative to the F_1 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 (1) 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 F_2 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-

pothesis 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; RISSE *et al.* 1989; HALAZONATIS 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.

We thank MIKE RILEY, DAWN FERRIS and WAN ZHANG for assisting in creation and maintenance of the deletion chromosomes. DOUGLAS SWANK analyzed the inheritance pattern of the borderline functional chromosome (#F599). TOM EICKBUSH, DANNA EICKBUSH and BILL BURKE gave advice on molecular procedures and TOM EICKBUSH kindly provided facilities. Comments from HANS BREEUWER and KENT REED improved the presentation of the results. This research was supported by grant #R01-HD24310 from the National Institute of Health.

LITERATURE CITED

ASHBURNER, M., 1989 *Drosophila. A Laboratory Handbook.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y.

BEUKEBOOM, L. W., K. M. REED and J. H. WERREN, 1992 Effects of deletions on mitotic stability of the Paternal Sex Ratio (*PSR*) chromosome from *Nasonia*. Chromosoma **102:** 20–26.

BEUKEBOOM, L. W., and J. H. WERREN, 1992 Population genetics of a parasitic chromosome: experimental analysis of *PSR* in subdivided populations. Evolution **46:** 1257–1268.

BEUKEBOOM, L. W., and J. H. WERREN, 1993 Transmission and expression of the parasitic Paternal Sex Ratio (*PSR*) chromosome. Heredity (in press).

BIESSMANN, H., and J. M. MASON, 1988 Progressive loss of DNA sequences from terminal chromosome deficiencies in *Drosophila melanogaster*. EMBO J. **7**: 1081–1086.

BLACKBURN, E. H., and J. W. SZOSTAK, 1984 The molecular structure of centromeres and telomeres. Annu. Rev. Biochem. **53**: 163–194.

BREEUWER, J. A. J., and J. H. WERREN, 1990 Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. Nature **346**: 558–560.

BROWN, J., J. A. CEBRA-THOMAS, J. D. BLEIL, P. M. WASSARMAN and L. M. SILVER, 1989 A premature acrosome reaction is programmed by mouse *t*-haplotypes during sperm differentiation and could play a role in transmission distortion. Development **106**: 769–773.

BROWN, S. W., 1960 Spontaneous chromosome fragmentation in the Armored Scale Insects (Coccoïdea–Diaspididae). J. Morphol. **106**: 159–185.

CROW, J. F., 1979 Genes that violate Mendel's rules. Sci. Am. **240**: 134–146.

CROW, J. F., 1988 The ultraselfish gene. Genetics **118**: 389–391.

DAVIDSON, B. E., and I. SAINTGIRONS, 1989 The *Escherichia coli* regulatory protein *Metj* binds to a tandemly repeated 8bp palindrome. Mol. Microbiol. **3**: 1639–1648.

DOOLITTLE, W. F., and C. SAPIENZA, 1980 Selfish genes, the phenotype paradigm and genome evolution. Nature **284**: 601–603.

EICKBUSH, D. G., T. H. EICKBUSH and J. H. WERREN, 1992 Molecular characterization of repetitive DNA sequences from a selfish B-chromosome. Chromosoma **101:** 575–583

GODFRAY, H. C. J., and P. H. HARVEY, 1989 Seed of destruction. Nature **337**: 210–211.

HABER, J. E., and P. C. THORBURN, 1984 Healing of broken linear dicentric chromosomes in yeast. Genetics **106**: 207–226.

HABER, J. E., P. C. THORBURN and D. ROGERS, 1984 Meiototic and mitotic behavior of dicentric chromosomes in *Saccharomyces cerevisiae*. Genetics **106**: 185–205.

HALAZONETIS, T. D., and A. N. KANDIL, 1991 Determination of the *c-MYC* DNA-binding site. Proc. Natl. Acad. Sci. USA **88**: 6162–6166.

HARTL, D. L., and Y. HIRAIZUMI, 1976 Segregation distorter, pp. 615–666 *The Genetics and Biology of Drosophila*, Vol. 1b, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

HOGGE, M. A. F., and P. E. KING, 1975 The ultrastructure of spermatogenesis in *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). J. Submicrosc. Cytol. **7**: 81–96.

HOWARD, C. A., G. R. GUMMERE, M. F. LYON, D. BENNETT and K. ARTZT, 1990 Genetic and molecular analysis of the proximal region of the mouse *t*-complex using new molecular probes and partial *t*-haplotypes. Genetics **126**: 1103–1114.

HUGHES-SCHRADER, S., and H. RIS, 1941 The diffuse spindle attachment of coccids, verified by the mitotic behavior of induced chromosome fragments. Exp. Zool. **87**: 429–456.

JAMES, A. C., and J. JAENIKE, 1990 "Sex Ratio" meiotic drive in *Drosophila testacea*. Genetics **126**: 651–656.

KLEIN, J., 1986 *Natural History of the Major Histocompatibility Complex*. Wiley, New York.

LEE, W., A. HASLINGER, M. KARIN and R. TJIAN, 1987 Activation of transcription by two factors that bind promotor and enhancer sequences of human metallothionein gene and SV40. Nature **325**: 368–372.

LYON, M., 1989 The genetic basis of transmission ratio distortion and male sterility due to the *t*-complex. Am. Nat. **137**: 349–358.

LYTTLE, T. W., 1991 Segregation distorters. Annu. Rev. Genet. **25**: 511–557.

MCCLINTOCK, B., 1941a The stability of broken ends of chromosomes in *Zea mays*. Genetics **26**: 234–282.

MCCLINTOCK, B., 1941b Spontaneous alterations in chromosome size and form in *Zea mays*. Cold Spring Harbor Symp. Quant. Biol. **9**: 72–81.

MCCLINTOCK, B., 1942 The fusion of broken ends of chromosomes following nuclear fusion. Genetics **28**: 458–463.

NUR, U., 1966 Harmful supernumerary chromosomes in a mealy bug population. Genetics **54**: 1225–1238.

NUR, U., 1977 Maintenance of a parasitic B chromosome in the grasshopper *Melanoplus femur-rubrum*. Genetics **87**: 499–512.

NUR, U., J. H. WERREN, D. EICKBUSH, W. BURKE and T. EICKBUSH, 1988 A "selfish" B chromosome that enhances its transmission by eliminating the paternal chromosomes. Science **240**: 512–514.

OLDS-CLARKE, P., and B. PEITZ, 1985 Fertility of sperm from *t*/+ mice: evidence that +-bearing sperm are disfunctional. Gen. Res. **47**: 49–52.

ORGEL, L. E., and F. H. C. CRICK, 1980 Selfish DNA: the ultimate parasite. Nature **284**: 604–607.

ÖSTERGREN, G., 1945 Parasitic nature of extra fragment chromosomes. Bot. Not. **2**: 157–163.

PEACOCK, W. J., and G. L. G. MIKLOS, 1973 Meiotic drive in Drosophila: new interpretations of the segregation distorter and sex chromosome systems. Adv. Genetics **17**: 361–409.

POWERS, P. A., and B. GANETSKY, 1991 On the components of segregation distortion in *Drosophila melanogaster*. V. Molecular analysis of the Sd locus. Genetics **129**: 133–144.

RISSE, G., R. MULLER, M. NEUBERG, K. JOOSSE and H. J. BRULLER, 1989 Asymmetrical recognition of the palindromic *Ap1* binding-site (*Tre*) by *Fos* protein complexes. EMBO J. **8**: 3825–3832.

RYAN, S. L., and G. B. SAUL, 1968 Post-fertilization effect of incompatibility factors in Mormoniella. Mol. Gen. Genet. **103**: 29–36.

RYAN, S. L., G. B. SAUL and G. W. CONNER, 1985 Aberrant segregation of *R*-locus genes in male progeny from incompatible crosses in Mormoniella. J. Hered. **76**: 21–26.

RYAN, S. L., G. B. SAUL and G. W. CONNER, 1987 Separation of factors containing R-locus genes in Mormoniella stocks derived from aberrant segregation following incompatible crosses. J. Hered. **78**: 273–275.

SANDLER, L., and E. NOVITSKI, 1957 Meiotic drive as an evolutionary force. Am. Nat. **91**: 105–110.

SAUL, G. B. II, P. W. WHITING, S. W. SAUL and C. A. HEIDNER, 1965 Wild-type and mutant stocks of Mormoniella. Genetics **52**: 1317–1327.

SEITZ, A. W., and D. BENNETT, 1985 Transmission distortion of *t*-haplotypes is due to interaction between meiotic partners. Nature **313**: 143–144.

SHAW, M. W., and G. M. HEWITT, 1990 B-chromosomes, selfish DNA and theoretical models: where next?, pp. 197–223 *Oxford Surveys in Evolutionary Biology*, Vol. 7, edited by D. FUTUYMA and J. ANTONOVICS. Oxford University Press, New York.

SILVER, L. M., 1985 Mouse *t* haplotypes. Annu. Rev. Genet. **19**: 179–208.

SKINNER, S. W., 1982 Maternally inherited sex ratio in the parasitoid wasp *Nasonia (Mormoniella) vitripennis*. Science **215**: 1133–1134.

TEMIN, R. G., 1991 The independent distorting ability of the *Enhancer of Segregation Distortion, E(SD)*, in *Drosophila melanogaster*. Genetics **128**: 339–356.

TEMIN, R. G., B. GANETSKY, P. A. POWERS, T. W. LYTTLE, S. PIMPINELLI, C-I. WU and Y. HIRAIZUMI, 1991 Segregation Distorter (SD) in *Drosophila melanogaster*: genetic and molecular analyses. Am. Nat. **137**: 287–331.

UEHARA, H., T. EBERSOLE, D. BENNETT and K. ARTZT, 1990 Submegabase clusters of unstable tandem repeats unique to the *Tla* region of mouse *t* haplotypes. Genetics **126**: 1093–1102.

WERREN, J. H., 1991 The Paternal-Sex-Ratio chromosome of Nasonia. Am. Nat. **137**: 392–402.

WERREN, J. H., U. NUR and D. EICKBUSH, 1987 An extrachromosomal factor causing loss of paternal chromosomes. Nature **327**: 75–76.

WERREN, J. H., U. NUR and C-I. WU, 1988 Selfish genetic elements. Trends Ecol. Evol. **3**: 297–302.

WHITING, A. R., 1967 The biology of the parasitic wasp, *Mormoniella vitripennis*. Q. Rev. Biol. **43**: 333–406.

WU, C-I., and M. F. HAMMER, 1991 Molecular evolution of ultraselfish genes of meiotic drive systems, pp. 177–203 *Evolution at the Molecular Level*, edited by R. K. SELANDER, A. G. CLARK and T. S. WHITTAM. Sinauer Associates Inc., Sunderland, Mass.

Communicating editor: D. CHARLESWORTH