A "Selfish" B Chromosome That Enhances Its Transmission by Eliminating the Paternal Genome

UZI NUR, JOHN H. WERREN, DANNA G. EICKBUSH, WILLIAM D. BURKE, THOMAS H. EICKBUSH

In the parasitic wasp, *Nasonia vitripennis*, males are haploid and usually develop from unfertilized eggs, whereas females are diploid and develop from fertilized eggs. Some individuals in this species carry a genetic element, termed *psr* (paternal sex ratio), which is transmitted through sperm and causes condensation and subsequent loss of paternal chromosomes in fertilized eggs, thus converting diploid females into haploid males. In this report the *psr* trait was shown to be caused by a supernumerary chromosome. This B chromosome contains at least three repetitive DNA sequences that do not cross-hybridize to each other or to the host genome. The *psr* chromosome apparently produces a trans-acting product responsible for condensation of the paternal chromosomes, but is itself insensitive to the effect. Because the *psr* chromosome enhances its transmission by eliminating the rest of the genome, it can be considered the most "selfish" genetic element yet described.

THE CONCEPT THAT SOME GENETIC elements have no function other than their own replication or transmission, in other words, that they are "selfish" or "parasitic" has attracted a great deal of interest in recent years (*1*). Possible examples of "selfish genes" include transposons, supernumerary (or "B") chromosomes, meiotic drive chromosomes, and sex ratio distorters. Here we describe a B chromosome that may be an extreme example of a selfish genetic element.

Nasonia vitripennis is a small (≈3 mm long) wasp that lays its eggs in the pupae of various fly species (*2*). Female wasps in this haplodiploid species normally alter the sex ratio among offspring by controlling the proportion of eggs that are fertilized (males develop from unfertilized eggs; females develop from fertilized eggs) (*3*). Both autosomal and extrachromosomal sex ratio variants exist in this species (*4, 5*). One of the most distinctive of these is the paternal sex ratio (*psr*) element. When females are crossed to *psr* males, they produce all-male or nearly all-male families that inherit the *psr* trait (*6*). In such crosses the absence of females is not due to mortality of the female embryos or failure of the sperm to fertilize the eggs; rather, the set of chromosomes of paternal origin becomes condensed after fertilization of the eggs, fails to divide, and is eventually lost (*7*). Because of haplodiploid sex determination, such haploid eggs develop into males. Use of genetic markers confirmed that all of the five paternal chromosomes are lost, which led to the conclusion that *psr* is extrachromosomal (not part of the normal chromosomal complement) during at least part of its transmission cycle (*7*). Thus, *psr* was believed to be either a cytoplasmic agent, such as a virus, or an unusual mobile genetic element. Attempts were therefore made to isolate *psr*-specific DNA.

To begin the analysis of *psr* at the molecular level a λ genomic library was constructed of total DNA from *psr* males (*8*). We reasoned that if *psr* is an extrachromosomal agent, such as a virus or transposable element, it would be present in many copies per cell. Thus, λ clones containing portions of this DNA agent would hybridize to total radioactively labeled DNA from *psr* males. Screening these same λ clones with total DNA from control males would reveal all those repetitive DNA sequences normally associated with the wasp genome. From a double screening of 6000 clones from the genomic library (*9*), 36 clones were selected that hybridized intensely to *psr* male DNA but not at all to control male DNA. These clones were regarded as a sublibrary of *psr*-specific clones.

Several clones from this sublibrary were selected for molecular analysis. Restriction enzyme analysis revealed that one of these clones, *psr2*, had a very simple structure. Seven restriction enzymes (Eco RI, Sst I, Kpn I, Sal I, Xba I, Xho I, and Bam HI) did not cleave anywhere within the 15–kilobase pair (kbp) insert of *psr2*, while Hind III cleaved the entire insert into fragments that were approximately 175 bp in length. This indicated that the entire insert was composed of approximately 85 tandem copies of a short DNA sequence. The repeat (Fig. 1 top) is 171 bp in length with no major internal direct or inverted repeats. Tandem repeats 170 to 185 bp in length, or approximately the length of DNA contained in one nucleosome, have been detected in a number of animal and plant species. Tandem repeats from a few nucleotides to hundreds of nucleotides in length are frequently referred to as satellite DNA and are usually associated with chromosomal regions that contain heterochromatin (*10*).

To determine the organization of the *psr2* repeat within the genome of *psr* males, the 171-bp repeat was used to probe genomic blots of DNA from *psr* and control males (Fig. 1 bottom). Consistent with our original screening of the genomic library, we found that when the DNA from control males was digested with Eco RI or Hind III (lanes c and d) no DNA complementary to the 171-bp repeat could be detected. The sensitivity of the hybridization in these experiments was such that at most only a few dispersed copies of the repeat could have gone undetected. When genomic DNA

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                                                        40
AAGCTTTTAT AAAAAGAACG CCGTCCTGTC GCCAACGACA
                                                        80
AGATGGCGGC CGGAGTGAGC TATTTGTATA GGCAGAATGC
                                                       120
GGGAGTCAAA AGTCTTGACT TTGGCTTACA CGCTTCTCTC
                                                       160
ACTCCAGTAG TAAGTATGAT CTTATACCCT TAAATTCCAG
                171
CTACATGCCA G
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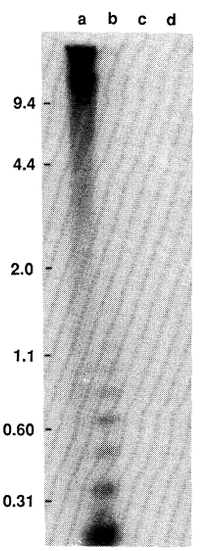

Fig. 1. (**Above**) Nucleotide sequence of the 171-bp repeat from clone *psr2*. Charon 35 clone *psr2* was digested with Hind III and ligated to sequencing vector m13mp19 (*21*). Two clones giving positive C-tests with each other were sequenced by the dideoxy method (*22*). The sequence of the two clones differed in only two positions. The clone not shown contained a C nucleotide at position 41 and an A at position 49. The Hind III restriction site has been underlined. (**Right**) Genomic blots of DNA from *psr* and control males probed with the 171-bp *psr2* repeat sequence. For each lane, 2 μg of DNA was digested, fractionated on a 1.0% agarose gel, and transferred to nitrocellulose. The blot was hybridized with the nick-translated 171-bp repeat isolated from clone *psr2*. Hybridization was conducted at 65°C in 0.6*M* saline [0.6*M* NaCl, 0.12*M* tris hydrochloride (*p*H 8), 4 m*M* EDTA], 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 25 m*M* sodium phosphate, 1% sodium pyrophosphate, 0.1% SDS, 10% dextran sulfate, and denatured calf thymus DNA (250 μg/ml). Final washing of the filter after hybridization was at 65°C in 0.015*M* saline and 0.1% SDS. DNA standards, in kilobases, are given at left. Lane a, *psr* male DNA digested with Eco RI; lane b, *psr* male DNA digested with Hind III; lane c, control male DNA digested with Eco RI; and lane d, control male DNA digested with Hind III.

Department of Biology, University of Rochester, Rochester, NY 14627.

from *psr* males was digested with Eco RI (lane a), most complementary DNA fragments were greater than 30 kb in size. This result could only be obtained if most copies of this repeat are in large tandem arrays. These arrays could either consist exclusively of the 171-bp repeat, as in clone *psr2*, or they could consist of these repeats interspersed with other repeated DNAs that do not contain Eco RI sites. When genomic DNA from *psr* males was digested with Hind III (lane b), all complementary DNA was 171 bp in length or a multiple of that length. This suggests that all copies of this repeat in the genome are highly conserved in sequence.

To determine the complexity of the *psr*-specific DNA, the 171-bp repeat was also used to rescreen the *psr*-specific sublibrary. The repeat hybridized to 25 of the 36 clones in the sublibrary, suggesting that it is probably the most abundant of the *psr*-specific DNAs. Partial analysis of the 11 clones in the sublibrary that did not hybridize to the *psr2* repeat has revealed that they contain at least two other classes of repetitive DNAs. Clones *psr18* and *psr22*, like *psr2*, are composed of tandem repeats approximately 175 bp in length. These repeats did not cross-hybridize with each other or with *psr2*. Of 3000 clones screened from the total *psr* library, 27 hybridized to *psr2*, 16 to *psr18*, and 8 to *psr22*. Thus, 1.7% of the genomic library of *psr* males is represented by these repeats (*11*). The *psr18* clone and several other clones from the *psr* sublibrary also contain stretches of nontandemly repeated sequences (*12*).

The conclusion that *psr* contained satellite-like DNA was not consistent with the hypothesis that *psr* was a virus or transposon, but suggested that it might represent a supernumerary chromosome, since satellite-like DNA is characteristic of heterochromatin from many eukaryotic chromosomes (*10*). On the basis of these findings, a cytogenetic analysis was carried out to determine whether *psr* was carried by a supernumerary (B) chromosome.

By means of standard cytogenetic procedures (*13*), 25 males were examined from stocks that did not carry the *psr* and 25 from crosses in which *psr* males were crossed to females from one of three different stocks that did not carry the *psr* (Lab II, R5-11, or G1-1A). All the males from the non-*psr* (control) stocks had the normal haploid number of five chromosomes. In contrast, 24 of the 25 sons of *psr* males carried, in addition to a haploid set of $n = 5$, a small extra chromosome (see Fig. 2). Thus, we conclude that *psr* is a B chromosome. The observation that one of the 25 sons of the *psr* males did not have the chromosome is not surprising, since *psr* is not transmitted to unfertilized eggs (*6*), and ovipositing wasps typically leave 5 to 15% of their eggs unfertilized under these experimental conditions.

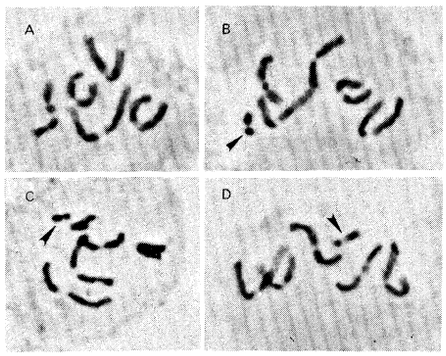

Fig. 2. Karyotypes of males in spermatogenesis (1240×). (**A**) A male with $n = 5$ from a line lacking *psr*. (**B** to **D**) Sons from crosses of *psr* males to females of a line lacking the *psr* trait. Nearly all the sons from such crosses carried the small B chromosome (arrows).

The B chromosome is submetacentric, and is much shorter than any of the regular chromosomes (see Fig. 2). From the relative size of the B chromosome and the haploid set, we estimate that the amount of DNA in the B chromosome is about 5 to 8% that of the haploid set. The appearance of the B chromosome suggests that it is composed mostly or wholly of heterochromatin, because in its degree of condensation it resembles the large blocks of pericentromeric heterochromatin that are present in all the regular chromosomes. This is most readily observed in early prophase. In addition to heterochromatin, however, the *psr* chromosome apparently contains one or more transacting genes that alter the normal pattern of condensation of the paternal chromosomes in a way that causes them to condense into a chromatin mass.

The observation that the B chromosome is highly heterochromatic is consistent with our molecular data showing satellite-like sequences in *psr*-specific DNA. Although prior studies have shown that B chromosomes share repetitive sequences with regular chromosomes (*14*), our findings demonstrate the existence of DNA sequences specific to a B chromosome.

The *psr*-containing chromosome could have been derived from either one of the five normal chromosomes of *N. vitripennis* or as a result of hybridization with another species. The presence of large tandem repeats (for example, *psr2*) that were absent in controls suggests that *psr* originated from an interspecific hybridization, or that these repeats were generated subsequent to *psr* divergence from one of the five *N. vitripennis* chromosomes. One possibility is that the *psr* chromosome originated after a cross between two strains (or species) of *Nasonia* that were incompatible. Such crosses have been shown to interfere with transmission of the chromosomes of paternal origin and to cause fragmentation of some of these chromosomes (*15, 16*). Moreover, Ryan and co-workers (*16*) were able to recover centromere-containing fragments from such crosses and to demonstrate that they are transmitted at a fairly high frequency through males (0.7–0.9), but are poorly transmitted through females (0.05–0.22). This asymmetric transmission can facilitate the evolution of such a fragment into a *psr* chromosome. However, for this to occur the fragment must incorporate one or more genes causing inactivation of the paternal set, and must become insensitive to the effect of such a gene or genes (*17*).

Supernumerary (B) chromosomes have been described in hundreds of animal and plant species; many of these chromosomes have been shown to be transmitted at a higher rate than expected from Mendelian inheritance and thus possess a "drive" (*18*). None, however, achieves its drive by actively destroying other chromosomes or by preventing the transmission of the host genome as does *psr*. The idea that B chromosomes that possess drive may be "parasitic" was first proposed by Ostergren in 1945 (*19*), long before the recent proposals that DNA may be "selfish" or "parasitic" (*1*). This idea was resisted for a long time, because of the difficulty of proving that these chromosomes do not confer a selective advantage on at least some of the individuals carrying them (*18*). In the case of the *psr* chromosome, however, there can be no question about any possible beneficial effects to the individuals carrying it, because males carrying this chromosome do not transmit any of their genes to their offspring. Indeed, because the *psr* chromosome enhances its own transmission by totally eliminating the rest of the genome, it may be considered the ultimate "parasitic" or "selfish" genetic element (*20*).

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8. DNA from *psr* males was partially digested with the restriction enzyme Sau 3A; 15- to 25-kb fragments isolated from a 5 to 20% sucrose gradient were ligated to the arms of Charon 35 [W. A. M. Loenen and F. R. Blattner, *Gene* **26**, 171 (1983)]. The recombinant DNA was packaged in vitro, plated, and amplified on strain KH802.
9. The Charon 35 library was plated at 1000 plaques per 150-mm petri plate. Two plaque lifts to nitrocellulose paper [W. D. Benton and R. W. Davis, *Science* **196**, 180 (1977)] were conducted of each plate. One set of filters was hybridized to 0.1 μg of *psr* male DNA labeled in vitro by nick-translation, while the second set of filters was hybridized to 0.1 μg of nick-translated control DNA. Hybridization was conducted at 65°C in 0.6*M* saline solution as described (Fig. 1). Final washes were at 65°C in 0.015*M* saline, 0.1% SDS.
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12. U. Nur, J. H. Werren, D. G. Eickbush, W. D. Burke, T. H. Eickbush, unpublished data.
13. Testes from *psr* and control male pupae were dissected out and kept for 1/2 hour in a solution containing 0.8% KCl and 0.2% colchicine. The testes were then rinsed in 70% ethanol, fixed in a 4:1 mixture of 95% ethanol, glacial acetic acid for 5 minutes, and stained and squashed in Lacto-aceto orcein.
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17. For the incipient *psr* chromosome to be selected to cause paternal genome loss, however, the species must have a female-biased sex ratio. The necessary conditions are $Bx > 1 - x$ where x = proportion of eggs fertilized, and B = proportion of sperm from *psr* males carrying the *psr* chromosome. See J. H. Werren, *J. Theor. Biol.* **124**, 317 (1987); S. W. Skinner, *Heredity* **59**, 47 (1987).
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20. Because *psr* causes the production of all-male families, it is also expected to have a negative effect on populations carrying it, either by lowering the growth rate of these populations, or by causing them to become extinct. Laboratory experiments confirm this (J. H. Werren, unpublished data).
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Scanning Tunneling Microscopy of recA-DNA Complexes Coated with a Conducting Film

M. AMREIN, A. STASIAK, H. GROSS, E. STOLL, G. TRAVAGLINI*

A link between scanning tunneling microscopy (STM) and conventional transmission electron microscopy has been established for biological material by applying STM on freeze-dried recA-DNA complexes coated with a conducting film. The topography of the complexes observed by means of STM revealed a right-handed single helix composed of about six recA monomers per helical turn.

SCANNING TUNNELING MICROSCOPY (STM) images structural and electronic properties of metal and semiconductor surface on an atomic scale (*1*). Imaging can be performed under atmospheric conditions, in insulating liquids, and even in electrolytes (*2–4*). The possibility of obtaining three-dimensional high-resolution images under various conditions makes STM an interesting technique for biological applications, with the ultimate hope of working in a biological environment that should preserve the biological sample in its native conformation. Exploratory STM efforts on DNA in vacuum (*5*) and on biological or organic matter under atmospheric conditions have been reported (*6–8*).

This report is on STM experiments on DNA and on complexes of DNA and recA, a protein involved in recombination, that were freeze-dried and coated with a conducting film. We have chosen recA-DNA complexes because their structure is a long, filamentous, well-characterized helix (*9, 10*) that is easy to distinguish on the support (Fig. 1). The periodicity is a signature to check reliability and reproducibility of imaging in different regions of the specimen. We used recA-DNA complexes formed in the presence of ATP[S] (the nonhydrolyzable analog of adenosine triphosphate). Image averaging and reconstruction (*9, 11*) from micrographs of negatively stained complexes and information from shadowed specimens show that this type of recA-DNA complex has a structure of a deeply grooved, right-handed helical filament of 10-nm diameter with about six recA monomers per helical turn. Very frequently, the complexes are not complete and have part of the DNA uncovered (Fig. 2).

Formed recA-DNA complexes (*9*) were purified from unbound recA protein on a Sepharose 2B column and subsequently adsorbed on platinum-carbon films (Pt-C) that were about 2 nm thick. The Pt-C film was evaporated on freshly cleaved mica with an electron gun. The tunneling current on this support was quite stable and not as noisy as on pure carbon films. The Pt-C films showed a granular structure with a typical grain size of 1.5 to 2 nm laterally and about 0.6 nm vertically. The adsorbing properties of Pt-C films for biological material were similar to those of carbon films that are routinely used for transmission electron microscopy (TEM) samples.

Freeze-drying followed by heavy-metal shadowing is an established TEM approach for routine structural studies of dehydrated biological specimens. Slow freeze-drying eliminates strong structural alterations due to effects of dehydration (*12*). To minimize film alterations after shadowing (such as oxidation or metal clustering caused by air contact), the metal films were stabilized with a relatively thick (5 nm) carbon-backing layer (*13*). Such a carbon coat would blur fine surface details when investigated with the tunneling microscope.

We found that at the right composition platinum-iridium-carbon films (Pt-Ir-C) remained three dimensionally stable after transferring to atmospheric conditions and that such films allowed, in contrast to pure carbon or Ir-C films, stable tunneling. In addition, Pt-Ir-C films showed a much smaller granularity than the standardly used Pt-C films, both in TEM and STM. Pt-Ir-C films were obtained by electron beam–induced evaporation of Pt-Ir cylinders (diameter 1.5 mm, 25% Ir) inserted in a carbon rod (diameter 2 mm). During evaporation the fraction of platinum decreased while the fraction of iridium and carbon increased. For stable tunneling a substantial content of metal was necessary, whereas for the three-

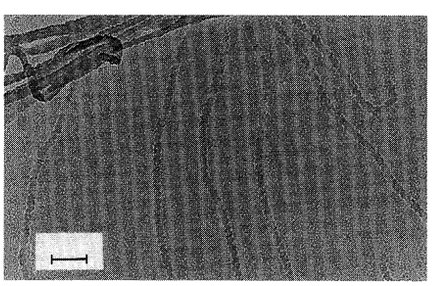

Fig. 1. TEM micrograph of freeze-dried recA-DNA complexes coated with a Pt-Ir-C film. The bar in the inset denotes a distance of 50 nm.

M. Amrein, A. Stasiak, H. Gross, Institute for Cell Biology, Swiss Federal Institute of Technology ETH Hönggerberg, 8093 Zurich, Switzerland.
E. Stoll and G. Travaglini, IBM Research Division, Zurich Research Laboratory, 8803 Rüschlikon, Switzerland.

*To whom correspondence should be addressed.