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PSR (paternal sex ratio) chromosomes: the ultimate selfish genetic elements

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Abstract

PSR (paternal sex ratio) chromosomes are a type of supernumerary (or B) chromosomes that occur in haplodiploid arthropods. They are transmitted through sperm but then cause loss of the paternal chromosomes (except themselves) early in development. As a result, PSR chromosomes convert diploid fertilized eggs (which would normally develop into females) into haploid males that carry a PSR chromosome. Because they act by completely eliminating the haploid genome of their 'hosts', PSR chromosomes are the most extreme form of selfish or parasitic DNA known. PSR was originally described in the parasitic wasp *Nasonia vitripennis* (Pteromalidae). A second PSR chromosome has been found in *Trichogramma kaykai*, an egg parasitoid from a different family of Hymenoptera (Trichogrammatidae). We argue that PSR chromosomes are likely to be widespread in haplodiploid organisms, but have so far gone under reported due to a paucity of population genetic studies in haplodiploids. We describe the two known PSR systems and related phenomena, and models indicating the conditions conducive to increase of PSR like chromosomes in haplodiploids.

Introduction

Parasitism is a ubiquitous feature of life and it is now widely accepted that parasites are common even within the genomes of organisms (Hurst & Werren, 2001). Examples of parasitic (or 'selfish') genetic elements include transposons, homing endonucleases, post-segregation killers, meiotic drive chromosomes, and B-chromosomes. They can generally be defined as heritable elements that have a replication advantage relative to other genetic elements within an individual's genome, but are either neutral or detrimental to the individual's survival and reproduction (Werren, Nur & Wu, 1988).

The first explicit characterization of a genetic element as being 'parasitic' was for a B-chromosome (Oestergren, 1945). B-chromosomes (reviewed in this volume) are non-vital 'extra' or supernumerary chromosomes and are widely found in plants and animals. Oestergren first proposed that these chromosomes were 'parasites' which had no function other than their replication and transmission. However, this view remained controversial for some time. It is now widely accepted that B-chromosomes are mostly parasitic, being maintained by replication advantages and imposing a fitness cost on the host (Beukeboom et al., 1998; Camancho, Sharbel & Beukeboom, 2000; He et al., 2000). However, some B-chromosomes may impart a fitness advantage, particularly at low copy number. Furthermore, it is often difficult to fully characterize the fitness consequences of B-chromosomes in ecological contexts where it is often difficult to measure fitness. Similar questions of fitness consequences occur with other parasitic genetic elements. That is, although elements such as B-chromosome, meiotic drive chromosomes and transposable elements have parasitic features and can be maintained by their replication advantages even when detrimental to the host (e.g., Hickey, 1982), it cannot always be established that such elements have no fitness advantages to hosts.

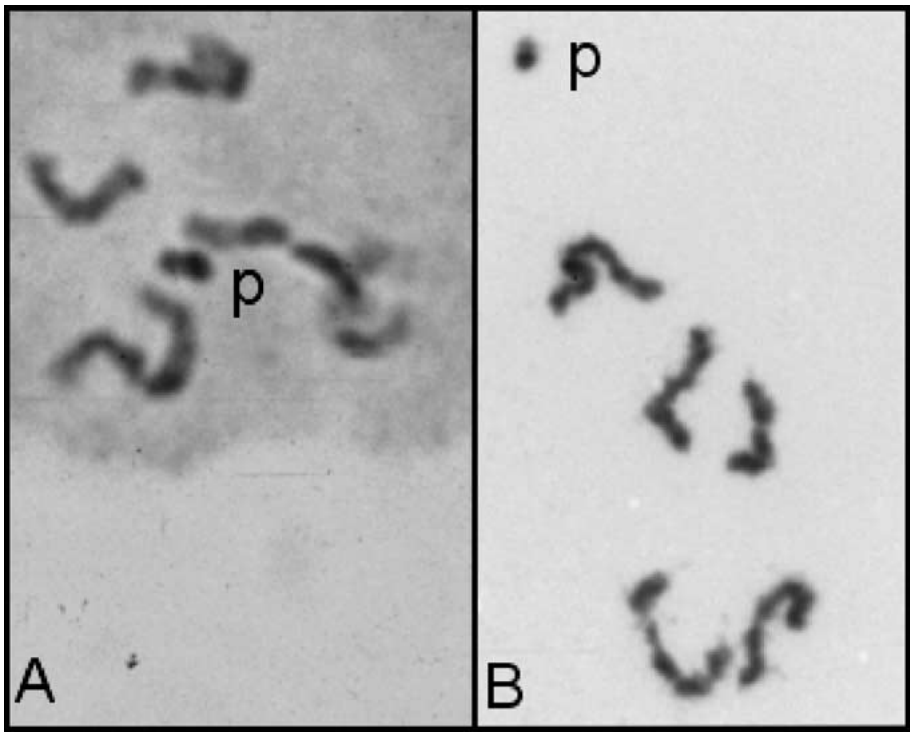

Figure 1. PSR chromosome (p) in (A) *Nasonia* spermatocyte cell and 5 A-chromosomes (see Nur et al., 1988 for methods), (B) *Trichogramma* somatic nucleus (reverse contrast image of DAPI stained nucleus, see Stouthamer et al., 2001 for methods).

The paternal sex ratio (PSR) chromosome is noteworthy as being a completely unequivocal example of a parasitic genetic element that has no fitness benefits to individuals that harbor it. As such it is a clear illustration of the concept that elements within the genome can evolve as parasites. PSR was first described in the parasitic wasp *Nasonia vitripennis*. It is a supernumerary B-chromosome found in some males of this species. As in other hymenoptera, *Nasonia* has haplodiploid sex determination; haploid males normally develop from unfertilized eggs whereas diploid females develop from fertilized eggs. This form of sex determination is essential for PSR-like chromosomes to evolve (see Figure 2).

PSR acts as follows: males who carry the chromosome produce functional sperm; however, after the sperm enters the egg the paternal (sperm) chromosomes (except PSR) form a dense chromatin mass at the first mitotic division, and are eventually lost. The result is a 'haploidized' embryo that develops into a functional male, and which carries the PSR chromosome (Figure 2). In the next generation, the chromosome set present in those males will also be destroyed following fertilization by the PSR-bearing sperm. Therefore, the PSR chromosome is responsible for the total destruction of the genetic material of its 'host', except for the PSR chromosome itself. Males who carry PSR have no genetic fitness and PSR therefore represents the most extreme example of a genomic parasite known for any organism.

PSR is an extreme example of parasitic DNA that disrupts normal sex determining mechanisms of its host. Other examples of parasitic sex ratio distorters include a number of microorganisms that feminize genetic males, induce parthenogenesis or cause male-killing, and meiotic drive sex chromosomes. Sex ratio distorters are of interest to evolutionary biologists because of they may play a role in the evolution of sex determining mechanisms (Cosmides & Tooby, 1980, for reviews see Werren & Beukeboom, 1998; Hurst, 1995). Studies of PSR chromosomes can therefore provide insights concerning the role of genetic conflict in shaping sex determination systems, and possible examples will be discussed further below. PSR chromosomes are also of interest because they disrupt early development in fertilized eggs, leading to selective loss of sperm-derived chromosomes. Understanding the mechanisms of PSR action could provide insights

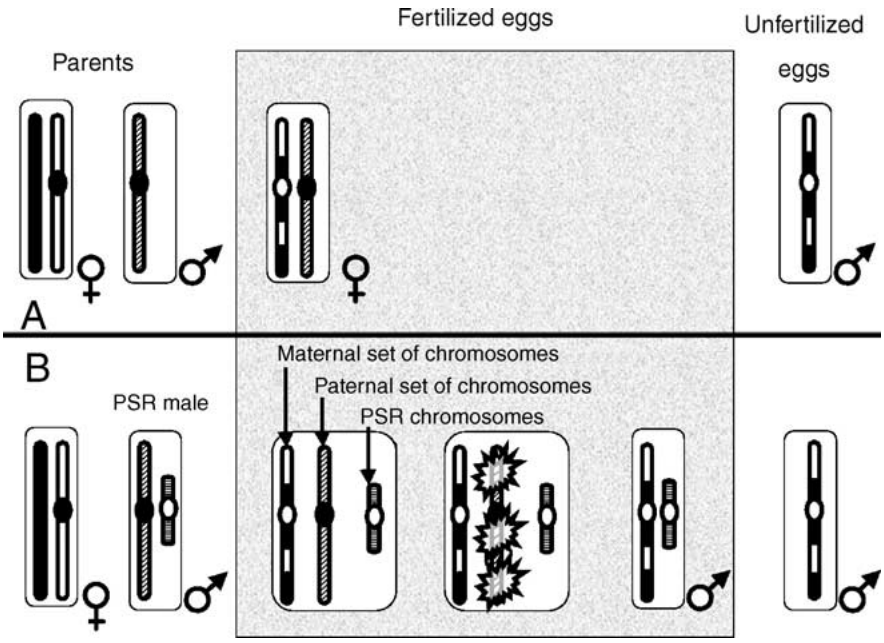

Figure 2. (A) Haplodiploid sex determination: Females are diploid while males are haploid. Fertilized eggs grow out to be diploid females while unfertilized eggs become haploid males. (B) When a female mates with a PSR chromosome carrying male her fertilized eggs grow out to be PSR males because the presence of the PSR chromosome in the sperm causes the destruction of the normal paternal set of chromosomes. The resulting PSR male carries the maternal set of chromosomes plus the PSR chromosome. Unfertilized eggs again give rise to haploid males.

about the mechanisms of paternal chromosome processing following fertilization. There are numerous pest haploidiploid insect species that cause many millions of dollars in damage. Examples include whiteflies, sawflies, seed chalcids, stinging bees and wasps, spider mites, and thrips (e.g., of species with haplodiploidy see Wrensch & Ebbert, 1991). PSR chromosomes could be of utility for biological control of pest haplodiploid species, either directly as a mechanism to suppress pest populations or indirectly as a vehicle for moving desirable genes across species boundaries.

Although an unambiguous and extreme example of parasitic DNA, until recently a PSR chromosome had only been described in *N. vitripennis* and therefore was believed to be a genetic anomaly. However, the discovery of a second PSR chromosome in the parasitic hymenopteran *Trichogramma kaykai* (Stouthamer et al., 2001) and PSR-like phenomena in *Encarsia pergandiella* (Hunter, Nur & Werren, 1993) raises the possibility that PSR and PSR-like elements are widespread and common in haplodiploid organisms. PSR-like elements have now been described in three different families of parasitic Hymenoptera. Given that detecting these effects requires population genetic studies and few haplodiploid species have been studied in the detail necessary to detect PSR elements in natural populations, we suspect that these chromosomes are more common in natural populations than previously assumed.

In this paper, we review the current status of research on PSR chromosomes. We consider their evolution, population dynamics, mechanisms of action and possible biological control applications, and discuss possible directions of future research.

Population dynamics of PSR chromosomes

The crucial feature of PSR dynamics is the fact that it is transmitted via sperm and therefore is only transmitted to fertilized eggs (Figure 2). In haplodiploid species not all eggs that give rise to offspring are fertilized. Unfertilized haploid eggs normally give rise to male offspring. PSR, in contrast, converts fertilized eggs that would have developed into diploid females into haploid males via destruction of the paternal chromosomes (except itself). As a result, PSR dynamics in random mating populations is very simple (Skinner, 1987; Werren, 1987; Werren & Beukeboom, 1993). Recall that males normally develop from unfertilized eggs and females from fertilized eggs in haplodiploids, but that PSR converts fertilized eggs into haploid

eggs. If we define e = frequency of PSR mated females, x = proportion of eggs that are fertilized, t = transmission rate of PSR to fertilized eggs in PSR male × female matings, and w = fitness of PSR males relative to normal males, then frequency in the next generation (e') is

$$e' = \frac{extw}{extw + 1 - x}. \quad (1)$$

This simply reflects the proportion of PSR males in the population (weighted by their fitness) relative to all the males in the population (derived both from PSR fertilized eggs and 'normal' males from unfertilized eggs). At equilibrium

$$e^* = \frac{x(tw + 1) - 1}{xtw}. \quad (2)$$

When transmission to fertilized eggs is 100% and PSR and normal males have equal fitness, this simplifies to

$$e^* = \frac{2x - 1}{x}. \quad (3)$$

This formula reveals the fundamental fact of PSR dynamics; PSR cannot invade a random mating population unless greater than 50% of the eggs are being fertilized. This is because in order for the PSR to increase in frequency among males in the population, it must produce a greater proportion of PSR males the proportion males produced in normal broods. Since PSR is transmitted during fertilization and normal males are produced by the absence of fertilization, it follows that more than 50% of the eggs must be fertilized for PSR to spread. The only other circumstance where PSR could increase in random mating populations is when PSR males have greater fitness than normal males ($w > 1$); however this seems unlikely and empirical studies suggest that PSR males have equal or reduced fitness relative to wild-type males (Beukeboom & Werren, 1993b).

Because 50% sex ratios are expected to evolve in random mating populations (Fisher, 1930), this means that PSR chromosomes cannot enter a population unless some other factor causes female-biased sex ratios (i.e., $x > 0.5$) (Figure 3(a)). Female-biased sex ratios favor the evolution of PSR-like elements in haplodiploid species. Among the conditions favoring female-biased sex ratios are: (a) population subdivision leading to partial inbreeding or local mate competition situations (Hamilton, 1967), (b) male-offspring being more costly to produce than females (Fisher, 1930), (c) worker control of sex ratios in social haplodiploid insects (Trivers & Hare, 1976) and (d) constrained sex ratios that can lead to female-bias such as in autoparasitoids and (e) presence of female-biasing sex ratio distorters such as heritable microorganisms. The last factor, female-biasing sex ratio distorters appears to be important in maintaining PSR chromosomes in two systems (*Nasonia* and *Trichogramma*) while constrained sex ratios may explain existence of the PSR-like element in the autoparasitoid *Encarsia pergandiella* (Hunter, Nur & Werren, 1993).

Population structure and inbreeding

In general, populations that are subdivided or have partial inbreeding select for female biased sex ratios. This situation would seem conducive to the spread of PSR-elements because greater than 50% of eggs are fertilized. However, both subdivided populations and inbreeding can select against a PSR element because these all-male producing elements suffer a transmission disadvantage in small local populations due to the shortage of available mates. For example, in species with partial sib-mating, males from PSR families have no available mates and PSR males in locally mating populations suffer from increased mate competition with their siblings (Werren & Beukeboom, 1993). Therefore, whether these population structures favor the evolution of PSR elements depends upon the trade-off between these two counterbalancing effects, selection for a greater fertilization proportion that favors PSR and a negative correlation between frequency of PSR and reduced availability of mates in local populations that is unfavorable for PSR.

Under a model (Stouthamer et al., 2001) where it is assumed that PSR transmission is 100%, fitness is equal in PSR and wild-type males and p proportion of females sib-mate while the remaining $1 - p$ mate randomly in the population at large, then equilibrium frequency of PSR-mated females is simply

$$e^* = \frac{2x - 1}{x} - p. \quad (4)$$

Thus, for any given proportion fertilized eggs, sib-mating reduces the expected frequency of PSR (Figure 3(a)). However, sib-mating also selects for a greater proportion of fertilized eggs (x). Under the situation of partial sib-mating, the expected equilibrium sex ratio (in the absence of PSR) under maternal sex ratio control is $x^* = (1 + p)/2$ for diploids (Maynard Smith, 1978; Taylor & Bulmer, 1980) and $x^* = [2 + 2p - p^2]/(4 - p)$ in haplodiploids, the difference being due to differences in relatedness to

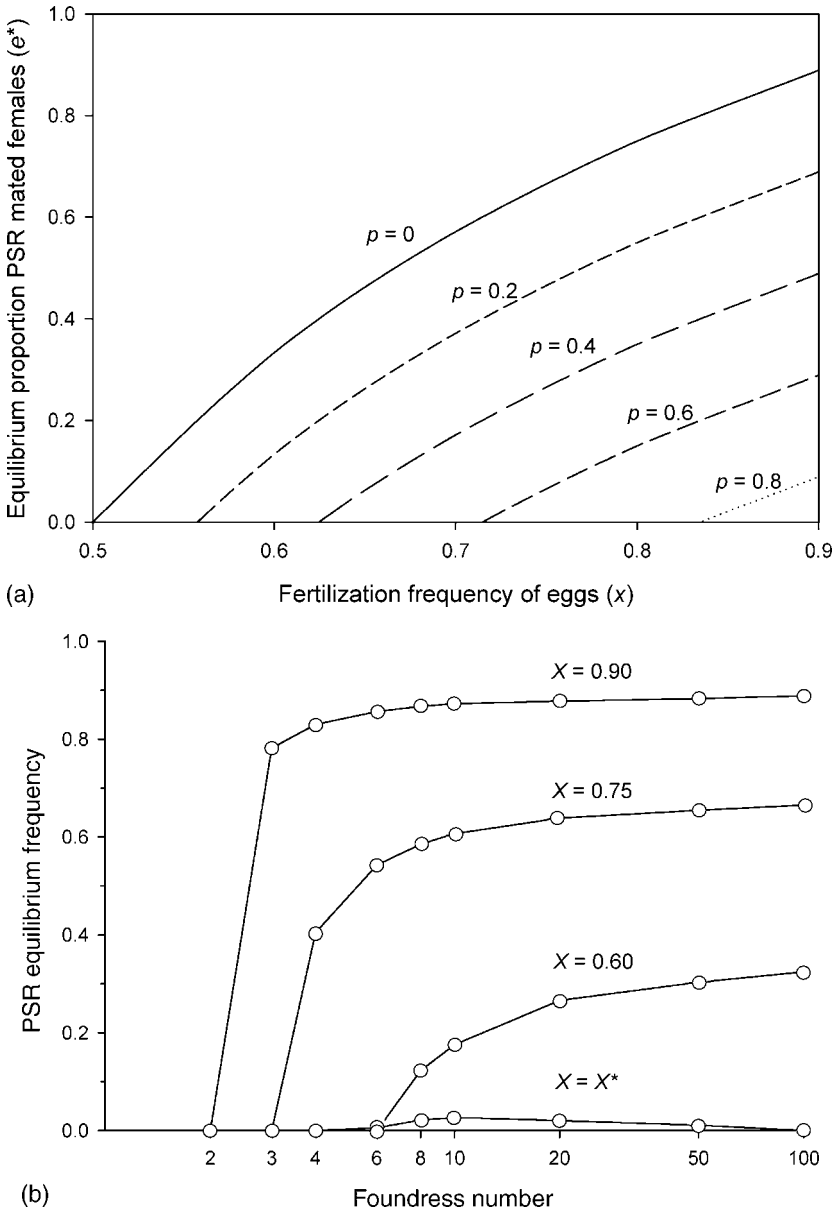

Figure 3. (a) Equilibrium PSR frequency in a random mating population with a given egg fertilization frequency. PSR cannot invade populations unless proportion fertilization above 1/2. Frequency of PSR also is the resulting reduction in population growth rate when PSR is present in a population at the indicated frequency. (b) Equilibrium frequency of PSR in populations divided into temporary (one generation) demes founded by *N* inseminated females (populations with a 'Hamiltonian' local mating structure. Equilibrium frequency depends upon the deme size and the fertilization proportion. As above, factors that lead to a greater bias in proportion fertilized eggs will increase frequency of PSR.

male and female offspring in haplodiploids under inbreeding (Suzuki & Iwasa, 1980). Substituting the latter into the above formula reveals an equilibrium frequency of PSR of $e^* = p[3 - 4p + p^2]/[2 + 2p - p^2]$. PSR can readily exist in populations with partial sib-mating. For example, in a species with 50% sib-mating, PSR is expected at frequencies of 23% and even at relatively low sib-mating rates such as 5%, PSR is expected at a frequency of 6.7%. Thus, the adaptive sex ratio effect of sib-mating is sufficient to allow establishment of a PSR chromosome, and even low levels of inbreeding can select for a PSR chromosome, indirectly through selection for a more female-biased sex ratio. Because partial inbreeding is

expected to be common in many haplodiploid species (except those with complementary sex determination), we expect PSR to be widespread in haplodiploids. The analyses for subdivided populations are more complex, and have only been derived for a 'Hamiltonian' population structure. This is one where females produce offspring in temporary (one generation) local demes each generation and mated female progeny then disperse to found new temporary demes (Werren & Beukeboom, 1993); males mate only locally. Under this population structure male progeny of a parent compete locally for mates and this local mate competition selects for female-biased sex ratios (Hamilton, 1967), but local mating also reduces effective transmission of PSR because of competition among the males of PSR families. Figure 3(b) shows the joint effects of sex ratio evolution and local mating structure on PSR. When the population is producing the expected equilibrium sex ratio for the particular deme size (Taylor & Bulmer, 1980), then PSR can only exist in an intermediate range of deme sizes (e.g., about 6–50 foundresses per deme) and then only at relatively low frequency. However, if egg fertilization rates are more biased than predicted by LMC theory, then PSR can achieve substantial frequencies (Figure 3(b)). Thus, other factors (e.g., sex ratio distorters) are needed for PSR to achieve appreciable frequencies in populations with an LMC structure, although PSR elements can exist at low frequencies.

Notice that the conditions are more restrictive than for the partial sib-mating population structure. The reason for this is that under LMC males always compete locally for mates, because they mate only in the local deme and all-male families suffer a fitness detriment for this. In contrast, under the partial sib-mating population structure, PSR siblings compete in the population at large for those females that do not sibmate, and therefore do not suffer an LMC disadvantage. They do lose access to that portion of the population that sib-mates, but this disadvantage is offset by the opportunities for mates in the population at large.

Size effects

In most haplodiploids, females tend to be larger than males. Therefore, in species where parents provision the young with food (e.g., solitary bees and wasps), female offspring will tend to be more expensive to produce than males and the resulting sex ratio is expected to be typically male-biased (Fisher, 1930; Charnov, 1982). PSR cannot readily exist under these circumstances. A correlated phenomenon occurs in some solitary parasitoid wasps where females tend to be placed in large hosts and males in small hosts. Generally, models of sex ratio evolution with variable host size predict that sex ratio will vary with availability of hosts, but tend to be equal or male-biased overall (Charnov et al., 1981; Werren, 1984). Thus, in general, host quality effects are not expected to select for PSR chromosomes, except in circumstances where they lead to female-biased population sex ratios.

Social hymenoptera

Can PSR elements evolve in social hymenoptera, due to selection on workers to produce female-biased sex ratios? One factor disfavoring PSR chromosomes in eusocial insects is colony level selection against those colonies founded by PSR mated females. Simply put, such colonies will produce few or no (female) workers and are likely not to survive. However, in social insects where either (a) females multiply mate (polyandry) or (b) colonies are maintained by multiple queens (polygyny), PSR elements may be able to exist. The situation would be similar to the occurrence of diploid males in polygynous colonies of some social insects (Ross, 1993).

Whether PSR can exist depends upon its negative effects on colony survival and productivity and the counterbalancing effects of female-biased sex ratios under worker control. Dynamics of PSR-like elements in social insects has not yet been explored theoretically. We can do an approximation here as follows. Assume that the queen has mated with 'm' different males and uses their sperm equally (or there are m queens in a colony who produce equal numbers of reproductives) then the equilibrium sex ratio under worker control is $x^* = r_f/[r_f + r_m]$ where r_f and r_m are, respectively, the relatedness of a worker to female and male reproductives produced in the colony; and $x^* = (2 + m)/(2 + 2m)$. The invasion criterion (condition allowing a PSR element to increase in the population when rare) is $x > 1/(w + 1)$ where w is the 'fitness' of a colony in which the queen has mated to 1 PSR male and $m - 1$ normal males (or one PSR mated queen occurs in a polygynous colony with $m-1$ normal mated queens). Fitness is relative to colonies with no PSR mated females.

Under these conditions, PSR can invade the population when $w > m/(2 + m)$. For colonies founded by single mated queens, PSR will not persist due to absence of female workers in colonies

founded by PSR mated queens. However, for higher frequencies of mating, the costs to a colony of having a single PSR mate may not be so large. For instance, PSR can invade the population if $w > 0.66$ for species with four mates per queen or 0.75 for colonies with six mates per queen. Therefore, there may be conditions where worker controlled sex ratios could favor PSR chromosomes, at least at low frequencies.

Split sex ratios (some colonies producing very female biased sex ratios while others produce very male-biased sex ratios) are predicted to evolve in some social hymenoptera due to asymmetries in relatedness between colonies (Boomsma & Grafen, 1990) and have been observed (Sundstrom, 1994). We do not know how 'split sex ratios' will affect the invasion conditions for PSR, but given that theory predicts that colonies with lower average relatedness (e.g., multiply mated queens) are expected to produce more male-biased sex ratios than those with higher average relatedness, split sex ratios due to asymmetries are expected to ameliorate against PSR elements. The analysis above does not consider differential costs of producing queens versus drones. If females are more costly to produce, then the overall numeric sex ratio is expected to be more male biased, which will select against PSR in social Hymenoptera, whereas if males are more costly, than the greater female bias in numeric sex ratio will favor PSR chromosomes. It should also be kept in mind that other causes for female biased sex ratios, such as presence of cytoplasmic sex ratio distorters, may be important in social hymenoptera but have not yet been extensively investigated. We fully expect the existence of such sex ratio distorters, and these will selectively favor invasion of PSR like elements (see below).

Sex ratio distorters

Cytoplasmic sex ratio distorters are likely to be important factors in the distribution of PSR elements in nature. Interactions between PSR and cytoplasmic sex ratio distorters have been explicitly modeled only in a few cases, by Werren (1987) for panmictic populations, Werren and Beukeboom (1993) for demic populations and Stouthamer et al. (2001) for partial sib-mating populations. In panmictic populations, simple models predict that a cytoplasmic factor producing 100% females will spread through a panmictic population, thus potentially driving the population to extinction due to a shortage of males. Introduction of PSR does not stabilize the system; PSR merely chases the cytoplasmic factor to fixation in the population, also driving the population to extinction. Clearly other factors are necessary to co-regulate a cytoplasmic sex ratio distorter. Under demic population conditions, a PSR chromosome can only regulate a cytoplasmic sex ratio distorter in populations with relatively small deme sizes, where PSR selects for non-distorting cytoplasmic genotypes (Werren & Beukeboom, 1993). Experimental population studies are also consistent with a regulation of cytoplasmic sex ratio distorters by PSR in highly demic populations but not in random mating populations, where both PSR and a cytoplasmic distorter achieve high frequencies (Beukeboom & Werren, 1993b).

Cytoplasmically inherited *Wolbachia* are known to cause thelytoky in several hymenopteran species; infected females produce female offspring with or without mating (Stouthamer et al., 1993; Stouthamer, 1997). In many infected species females will not mate; however, in some other species females will mate with males, and utilize the sperm chromosomes to produce sexual offspring. Stouthamer et al. (2001) have shown that a PSR element can attain high frequency under these circumstances, due to the relatively high numbers of virgin females (mates) caused by the thelytoky-inducing bacterium. Furthermore, the model shows that PSR can regulate the microorganism, preventing it from going to 100% in the population.

In summary, theoretical studies indicate that PSR elements can exist in haplodiploid populations when greater than 50% of the eggs are fertilized. Partial inbreeding and population subdivision can permit PSR to occur in populations indirectly, due to selection for female-biased sex ratios. A second important factor favoring existence of PSR elements is presence of cytoplasmically inherited female-biasing factors. In general, the PSR elements are expected to occur at low frequencies, with higher incidences occurring only when female distorting factors achieve high frequencies in the population. Under some circumstances, PSR can regulate the frequency of cytoplasmic female-biasing elements. Evidence is accumulating that female-biasing cytoplasmically inherited microorganisms are common in insect species, particularly in haplodiploids (see Hurst, 1995; Werren, 1997; Stouthamer et al., 1999). Therefore, we expect PSR to also be widespread in haplodiploids, and commonly associated with female-biasing sex ratio distorters. PSR elements may possibly occur

in social hymenoptera under conditions of multiple mating or multiple queen colony structure and worker influence over reproductive sex ratios, or other factors that lead to greater than 50% egg fertilization among reproductive progeny.

PSR of *Nasonia*

The PSR chromosome of *Nasonia* has been studied in some detail. It was first discovered in 1981 (Werren Skinner & Charnov, 1981) as a paternally transmitted sex ratio distorter that caused all-male families, but was not identified as a B-chromosome until later (Nur et al., 1988). Cytogenetic studies revealed that PSR induces all-male families by causing improper condensation of the paternal chromosomes at the first mitosis in fertilized eggs (Werren, Nur & Eickbusch, 1987; Reed & Werren, 1995).

The *Nasonia* PSR is a submetacentric B-chromosome estimated to represent about 5.7% of the nuclear genome of carrier males (Reed, 1993). Based on estimates of the haploid genome size of *Nasonia* of 250 MB (Rasch, Cassidy & King, 1975), this gives an estimated size of PSR of 15.1 MB. PSR is highly heterochromatized and contains large quantities of tandem repetitive DNA characteristic of heterochromatic regions (Eickbush, Eickbush & Werren, 1992; Reed et al., 1994). The chromosome generally remains condensed through the cell cycle. C-banding of prometaphase spermatocytes revealed a secondary constriction on PSR that could be indicative of a small euchromatic region. However, attempts to isolate regions of single copy DNA by examining regions at the end of tandem repetitive DNA on the chromosome were unsuccessful. These studies did, however, yield information on transposable elements present on PSR (McAllister, 1995; McAllister & Werren, 1997b).

The chromosome harbors a number of families of tandem repetitive DNA, ranging in repeat size from approximately 120–250 bp. Some repeat families are unique to PSR and absent from the A-chromosomes of *Nasonia*, whereas others are shared between PSR and the three *Nasonia* species and closely related genus *Trichomalopsis* (Eickbush, Eickbush & Werren, 1992; Beukeboom, Reed & Werren, 1992). There is no evidence that these sequences are transcribed to RNA. The repeats are found in very large arrays on the chromosome, however, certain families may occur in multiple blocks on the chromosome (Beukeboom, Reed & Werren, 1992; McAllister, unpublished). One proposal is that tandem repeats act as a 'sink' to bind away proteins necessary for proper processing of the paternal chromosomes (described in more detail later).

The tandem arrays present on PSR have been used to study evolution of tandem arrays in general. An analysis of junctions between two families of tandem arrays on PSR implicated palindromic regions within the repeats as sites of unequal chromatid exchange (Reed et al., 1994), one mechanism believed to result in amplification of tandem repetitive DNA. Further studies of the junctions between tandem arrays and transposon insertions indicated suppression of unequal exchange in the immediate vicinity of insertion sites (McAllister & Werren, 1997b). Observations from the study also led to a model proposing that transposable elements will accumulate at the ends of arrays, and into 'islands' within tandem arrays due to unequal chromatid exchange following transposon insertions.

NATE (*Nasonia* transposable element) is a family or retrotransposable elements, related to Gypsy/Ty3 elements and found in *Nasonia* and closely related genera. McAllister and Werren used NATE to demonstrate strong conservation in amino acid sequence in the reverse transcriptase domain, and also provided data showing that individual transposon insertions accumulate mutations like 'pseudogenes', whereas lineages of actively transposing elements show sequence conservation at non-synonymous sites. Further study of NATE elements provides evidence on the origin of PSR by interspecific hybridization. Detailed phylogenetic studies of NATE family transposons on related wasp species showed that PSR specific transposable elements were most related to transposons found in wasps of the genus *Trichomalopsis* (McAllister, 1995; McAllister & Werren, 1997a, b). Therefore, the PSR chromosome must have been in its evolutionary past associated with this genus.

Genetic studies of *Nasonia* PSR have been geared towards determining the mode of action of this chromosome. Deletions of PSR using radiation and *Wolbachia*-induced cytoplasmic incompatibility can result in 'non-functional' PSR – chromosomes which have lost the ability to induce paternal genome loss (PGL). Function tentatively maps to the short arm of PSR, although there may be functional redundancy on PSR (Beukeboom, Reed & Werren, 1992; McAllister et al., unpublished). PSR has also been moved successfully into two species closely related to *N. vitripennis*, i.e. *Nasonia giraulti* and *N. longicornis* (Dobson & Tanouye, 1998b; Beukeboom & Werren,

2000). In these species the chromosome behaves in a manner indistinguishable from its behavior in *N. vitripennis*. Dobson and Tanouye (1998a) showed that the PSR chromosome is capable of inducing complete PGL in the sperm of diploid males, indicating an excess of PGL capability on the chromosome. They also used crosses between PSR males and triploid females, which generated diploid and haploid males, to argue that sex determination in *Nasonia* (and other chalcids that lack complementary sex determination) involves genomic imprinting of the paternal genome. However, this interpretation is not supported by some other evidence (Braig et al., in press), including the production of females from unfertilized eggs in *Trichogramma* (Stouthamer, 1997), and the production of gynandromorphs from unfertilized eggs in *Nasonia* (Werren, unpublished).

The population biology of PSR has been studied extensively in the laboratory and field. In the laboratory the transmission rate of PSR to its offspring is very high (Beukeboom, 1994). Approximately 90% of the males transmit PSR to 100% of the fertilized eggs of their mates. Of the remaining 10% daughters were rarely found among the offspring of PSR fathers, these daughters generally were free of the PSR chromosome indicating that apparently not all sperm of these PSR males contained the PSR chromosome (Beukeboom & Werren, 1993a). Experimental population studies using panmictic and subdivided structures show that PSR achieves equilibrium frequencies generally predicted by the population structure and egg fertilization level (as described above). Interactions were found between PSR and the MSR element, a factor that causes fertilization of nearly 100% of eggs and therefore all-female families. Basically, PSR could select against an MSR element in highly subdivided populations; however this was an accidental finding of the population experiments and interactions between PSR and MSR have not been very thoroughly explored.

Extensive field studies have been conducted to delineate the distribution of PSR in nature. So far, the PSR chromosome has only been found populations of *N. vitripennis* in the Great Basin area of North America, including sites in the US states of Utah, Idaho and Wyoming. While not all areas have been sampled as intensively as the areas mentioned above, some intensively sampled areas such as upstate New York have failed to produce a single PSR. In the areas where PSR occurs it is uncommon, typically 1–5% of the females sampled from the field had mated with a PSR male (Skinner, 1983; Beukeboom & Werren, 2000). *Nasonia* lays its eggs gregariously in fly pupae and upon emergence brother sister mating occurs. While females are winged, males have very short wings and are unable to fly. Consequently males are limited in their mating opportunities to the females emerging in their natal patch. This makes PSR very dependent on large patches where the PSR males can mate with virgin females emerging from other fly pupae. Given the population subdivision in *N. vitripennis*, where many wasps may emerge in patches with only the offspring of a single mother, PSR is not expected to reach very high frequencies in *Nasonia* (Beukeboom & Werren, 1992; Werren & Beukeboom, 1993), except in the presence of high frequencies of the MSR element. However, field sampling did not show a correlation between MSR frequency and PSR frequency from a relatively small set of sampled populations (Beukeboom & Werren, 2000).

Theory predicts that a PSR chromosome should strongly select for autosomal repressors to its action. The reason for this is simple, PSR eliminates transmission of nuclear genes from males who carry it. However, screening of natural populations have so far not detected repressors to PSR action (Werren, unpublished). It is possible that the low frequency of PSR in nature does not create selective forces sufficient for repressor selection.

The *Nasonia* PSR has been used to investigate several basic biological questions, including sex determination in Hymenoptera (Dobson & Tanouye, 1998a), molecular evolution of transposable elements (McAllister & Werren, 1997b), origin and evolution of B-chromosomes (McAllister & Werren, 1997a; Beukeboom, Reed & Werren, 1992; Dobson & Tanouye, 1998b), dynamics and evolution of tandem repetitive DNA (Reed et al., 1994; McAllister & Werren, 1999) and the role of genetic conflict in sex ratio evolution (e.g., Beukeboom & Werren, 1992; Werren & Beukeboom, 1993). However, the fundamental question of how PSR induces paternal genome loss and how the chromosome evolved this ability has yet to be resolved.

PSR of *Trichogramma*

A PSR chrosomosome has also been discovered in *Trichogramma kaykai*, a minute egg parasitoid that is found in the Mojave Desert, where it parasitizes eggs of the lycinid butterfly *Apodemia mormo* (Stouthamer

et al., 2001). Female wasps generally oviposit 3–5 wasp eggs per host. As predicted by the local mate competition theory (Hamilton, 1967) a mother allocates generally one unfertilized (i.e., male) egg and 2–4 fertilized (i.e., female) eggs to a host. Upon emergence the male mates with his sisters. The frequency of this brother–sister mating is estimated to be between 55 and 65% of the females (Stouthamer & Kazmer, 1994). In addition to the PSR chromosome, *T. kaykai* populations also harbor another sex ratio distorter, that is, the parthenogenesis inducing *Wolbachia* (Stouthamer, 1997). Infection with this *Wolbachia* enables infected females to produce exclusively female offspring, both from unfertilized and from fertilized eggs. Approximately 4–26% of all *T. kaykai* females are infected with this bacterium. PSR was discovered in this species when other hypotheses on how the *Wolbachia* infection frequency was kept at the low level were rejected and modelling showed that a PSR like factor could maintain the infection frequency at the low levels. PSR is very common in these populations and approximately 10% of all males carry the PSR chromosome. PSR was detected in all sampled populations (Stouthamer et al., 2001).

The *Trichogramma* PSR shows many similarities with the *Nasonia* PSR. It is also a small supernumerary chromosome, being less than 10% of the genome (Stouthamer & van Vugt, unpublished), as measured by the chromosome length. Eggs fertilized with PSR sperm develop into males, again carriers of the PSR, while the other chromosomes of the father are destroyed. Eggs infected with *Wolbachia* and fertilized with PSR sperm develop into infected PSR carrying males (Stouthamer et al., 2001).

In cytogenetic studies of the fertilization process (van Vugt et al., in preparation), the mechanisms of chromosome destruction and its timing are remarkably similar to that of *Nasonia* (Reed & Werren, 1995). In the first mitotic division of the fertilized egg the paternal chromosomes form a dense mass (Paternal Chromosome Mass) that remains visible in the eggs for at least six mitotic divisions. The transmission rate of the PSR from father to his offspring is very high (Stouthamer, unpublished). Little is known yet about the presence of genes on the PSR chromosome, no studies have as yet determined the sequences of the repeats on this chromosome. However, primers specific for the *Nasonia* PSR repeats were tested, but they failed to amplify products from genomic DNA of a *Trichogramma* PSR (Stouthamer, unpublished). Although the cytological mode of action of these chromosomes is very similar they probably contain different sequences, and almost certainly represent independent evolutionary origins.

Similar to the situation in *Nasonia* it appears to be very simple to transmit the PSR of *Trichogramma* to other *Trichogramma* species in the lab. We have succeeded in entering PSR into *Trichogramma deion*, this species is closely related to *T. kaykai*, and also in the taxonomically more distant species *Trichogramma sibericum* (Stouthamer & van Vugt, unpublished). The PSR was entered in these other populations by forcing interspecific matings between *kaykai* PSR males and parthenogenesis inducing *Wolbachia* infected females of the recipient species. In the recipient species the PSR acts similarly as it does in *T. kaykai*. The dynamics of the PSR frequency among males has been modeled in populations with varying fertilization rates and levels of brother–sister matings (sib-matings) by Stouthamer et al. (2001). The high prevalence of the PSR in *T. kaykai* is mainly caused by the high infection frequency of parthenogenesis-inducing *Wolbachia*. But even without the *Wolbachia* infection the PSR should be able to maintain itself in the *T. kaykai* populations, because the inherent female-biased sex ratios in this species due to sib-mating. Without the *Wolbachia* infections, the PSR frequency is predicted to be at low levels, comparable to those found in *N. vitripennis* (Beukeboom & Werren, 2000).

The PSR-like element of *E. pergandiella*

In addition to the two PSR chromosomes already described, a ‘PSR-like’ element has been discovered in the autoparasitoid wasp *E. pergandiella* (Hunter, Nur & Werren, 1993). *E. pergandiella* has an unusual biology. The normal reproduction in *E. pergandiella* is as follows. Females develop as primary internal parasitoids of whiteflies. Males, however, develop as parasitoids of females of their own or other parasitoid species, and therefore develop as hyperparasitoids. This unusual reproduction method is characteristic for wasps of the genus *Encarsia* and is known as ‘autoparasitism’ or heteronomous hyperparasitism (Walter, 1983; Hunter & Woolley, 2001). Normally males can only develop as hyperparasitoids, and male eggs oviposited in whiteflies fail to develop.

Hunter, Nur & Werren (1993) discovered a non-mendelian factor in a population in upstate New York that induces paternal genome loss in fertilized eggs, converting them into haploid males that developed

directly on whitefiles (i.e., as primary rather than the normal hyperparasitoid males). Cytological examination failed to detect a B-chromosome; however, the trait was transmitted paternally from primary males to about 28% of the male offspring of their mates. Since the male offspring do not inherit chromosomes from the 'father', this result indicates that the element is either extrachromosomal or a very small B-chromosome that was not visible under the cytological methods employed. X-ray studies of primary and hyperparasitoid males showed that the integrity of the paternal set of chromosomes was not needed for the production of primary males. When normal females were mated with irradiated primary males they produced some primary male offspring, while females mated to irradiated hyperparasitoid males produced no offspring at all on whitefly hosts. The authors conclude that the most likely explanation for the PSR-like behavior is induced either by a transposable element or by a paternally inherited virus. Nevertheless, similarities to PSR chromosomes are clear – induction of paternal genome loss by overcondensation of the paternal set at the first mitosis, and paternal inheritance of the factor.

Why is the PSR-like element selectively favored? Because of the constraints of autoparasitism, normal male offspring are only produced as hyperparasitoids of developing parasitoids. As a result, skewed population sex ratios occur at certain times due to the abundance of whitefly hosts relative to parasitoid hosts and the hard constraint that haploid unfertilised eggs cannot develop on primary hosts. The PSR-like element apparently circumvents this problem, because fertilized eggs do develop on primary hosts, even if they become male due to paternal genome loss. As a result, PSR-like elements will be favored in such a system whenever the proportion of fertilized eggs (primary hosts parasitised) exceeds 50% . We therefore expect PSR elements in other systems where hard constraints result in fertilization of greater than 50% of eggs.

The finding of the two cases of PSR in both *Nasonia* and *Trichogramma*, plus the presence of an unknown, but non-B-chromosome factor in *E. pergandiella* (Hunter, Nur & Werren, 1993) indicates that these factors may be more common in Hymenoptera than we have previously thought. In general sex ratio distorters that cause all-male broods in Hymenoptera are easily overlooked. Virgin females also produce all-male broods and it is not uncommon for researchers to ascribe the presence of all-male families in a hymenopteran species to failure of a certain proportion of females to mate. Detection of PSR elements requires that the males from these all-male families be tested for inheritance of the tendency to produce all-male families. If males from all-male families have a higher tendency to produce all-male progeny than do males from mixed sex ratio families, then a PSR element is strongly implicated. The reason for this is simple, males do not transmit nuclear genes (except PSR chromosomes) to the male offspring of their mates. Some systems with incidences of all-male producing females in natural populations that are attributed to virginity may actually be due to presence of PSR chromosomes (Godfray, 1990; Godfray & Hardy, 1991).

Mechanisms of action of PSR

The mechanisms by which PSR induces PGL remain a mystery. There are two essential features – PSR induces hypercondensation and loss of the paternal A-chromosomes and PSR is immune to this hypercondensation and is successful incorporated with the maternal set at mitosis. Cytologically, the appearance of PGL is remarkable similar for the PSR chromosome of *Nasonia*, *Trichogramma* and the PSR-like element in *Encarsia*. In *Nasonia*, abnormalities are first observed following fertilization of the egg by PSR-bearing sperm, prior to the first mitosis (Reed & Werren, 1995). Following fertilization, the paternal pronucleus migrates to the interior of the egg and comes to lie adjacent to the maternal pronucleus. At this point, the paternal pronucleus from PSR-bearing sperm shows abnormalities in shape (e.g., is comma shaped as opposed to the normal spherical shape). This observation suggests that the lesion(s) induced by PSR occur between entry of the sperm into the egg and the breakdown of the nuclear envelope prior to commencement of the first mitotic division. There are a number of steps involved to convert the sperm to a pronucleus that is capable of participating in development. These include removal of the sperm nuclear envelope; decondensation of the sperm chromatin; replacement of sperm chromosomal proteins with maternally supplied histone; assembly of a nuclear envelope, lamina, and matrix; and chromosome replication and condensation (Poccia & Collas, 1996). PSR may either block a specific step in this process (e.g., histone replacement) or alternatively may act by disrupting the timing of conversion of the male pronucleus, in either case resulting in disruption of

paternal chromatin condensation at the first mitosis (Reed & Werren, 1995).

The paternal chromosomes form a dense chromatin mass prior to the first metaphase, and this mass tends to remain on the equational plane as the haploid maternal set and PSR undergo apparently normal mitosis. Hoechst staining shows that chromatin in the paternal pronucleus replicates prior to the first mitosis (as expected), but does not replicate in later mitotic cycles. The chromatin mass does not participate in subsequent mitoses, remains condensed during interphase, and persists at least until cellularization of the syncytial blastoderm when it is found near the center of the egg with the yolk nuclei. The resulting embryo is haploidized and develops into a male.

Interestingly, PSR-induced PGL is cytologically similar to PGL induced by the endosymbiont *Wolbachia* (Reed & Werren, 1995; Callaini, Dallai & Riparbelli, 1997). *Wolbachia* are a widespread group of intracellular bacteria found in over 15% of insect species, as well as in nematodes, mites and crustaceans (Werren, 1997; Stouthamer, Breeuwer & Hurst, 1999 for reviews). Many *Wolbachia* induce a sperm egg incompatibility that results in disruption of the paternal chromosomes at the first mitosis, akin to that induced by PSR. This has led to the interesting suggestion that PSR may have acquired its ability to induce PGL via genetic exchange with the *Wolbachia* present in *Nasonia* and related wasps. However, the PGL or PSR and *Wolbachia* have been compared in *Nasonia* and differ cytologically (Reed & Werren, 1995). The *Wolbachia*-induced paternal chromatin mass is less compacted and is often observed to shear during mitosis, with portions entering the daughter nuclei. As a result, centric fragments of paternal chromosomes (de novo B-chromosomes) can occasionally survive *Wolbachia*-induced PGL and be transmitted to future generations (Ryan, Saul & Conner, 1985, 1987; Perfectti and Werren, 2001; Perrot-Minnot & Werren, 2001). The joint phenotype of PSR- and *Wolbachia*-induced PGL have been investigated by crossing *Wolbachia* infected PSR males to uninfected females in *N. vitripennis* (Reed & Werren, 1995). Cytologically, these appear most like PGL induced by *Wolbachia*. Furthermore, the PSR chromosome is also eliminated in CI crosses, indicating that it is not immune to CI and that the two elements employ different mechanisms of PGL.

Two basic molecular models have been proposed for PSR induced PGL. Either PSR produces a product that modifies (e.g., imprints) the paternal chromosomes or PSR acts a 'sink' to bind away some product (e.g., a chromatin binding protein) necessary for normal paternal chromosome processing in the ferilized egg. The sink model has a certain appeal because it can account by the same process for the two basic features of PSR, ability to disrupt the paternal A-chromosomes and immunity from the disruption. Induction of both PGL and immunity by the same process is evolutionarily more likely because both are required for a PSR chromosome to persist (see origins).

Modification of the A-chromosomes by PSR could occur during spermatogenesis or subsequently in the fertilized eggs. Given the short period of time (less than 1 h) between entry of the sperm into the egg and the first mitosis (Tram & Sullivan, 2000) and transcriptional inactivity of paternal pronuclei in nearly all organisms, the latter scenario requires PSR to function as a sink rather than as a transcriptionally active unit.

In general very few active genes appear to be present on B-chromosomes. A large part of the DNA on B-chromosomes consists of repeated DNA sequences. These repeated sequences are not just a single repeat but can consist of different families of repeats as has been shown in *Nasonia*. The large number of repeats present on B-chromosomes is most likely responsible for them being heterochromatized (Bigot, Hamelin & Periquet, 1990). It has also been suggested that the repeats may function as a target for nuclear proteins (Charlesworth, Sniegowski & Stephan, 1994). This may be one of the mechanisms through which B-chromosomes acquire preferential inclusion in gametes. Ribosomal genes are also often found on B-chromosomes, however these appear not to be active (Donald et al., 1997). Finally B-chromosomes also accumulate transposable elements. The large number of elements found on these chromosomes is most likely associated with their lack of recombination, as is particularly the case for PSR in *N. vitripennis*. Molecular genetic studies have revealed an abundance of repetitive DNA, both tandem and dispersed, on the *Nasonia* PSR, some of which are unique to the PSR chromosome (Eickbush, Eickbush & Werren, 1992, Reed et al., 1994, McAllister, 1995; McAllister & Werren, 1997a, 1999). It has been proposed that such repeats could act as a 'sink' to bind away a protein needed for normal chromosome processing in the fertilized egg.

As mentioned, deletions have been used to analyze structure and function of the *Nasonia* PSR. Basically, deletion of large regions can result in 'non-functional' PSR chromosomes that are no longer able to cause

PGL. Mapping studies indicate that PSR function may localize to the short arm of this metacentric chromosome, but are not completely conclusive in this regard (McAllister et al., unpublished). One difficulty has been the paucity of molecular markers in these earlier studies. Additional molecular markers (e.g., AFLPs or microsatellites) may produce a sufficient density markers for fine-scale mapping of the functional domains on PSR. In addition, molecular cytogenetic approaches could prove very useful in elucidating the steps of paternal chromosome processing that are disrupted in PSR-fertilized eggs. A combination of genetic, molecular and cytological approaches will be important in determining how PSR chromosomes disrupt paternal chromosomes and are themselves immune from this action.

Origins and evolution of PSR chromosomes

Where do PSR chromosomes come from? The B-chromosomes in many species are thought to have originated from the normal A-chromosomes of the same species. In contrast, evidence indicates that the PSR chromosome of *N. vitripennis* entered *Nasonia* by hybridization with a closely related genus of parasitoids (*Trichomalopsis*). It is yet to be determined whether the *Nasonia* PSR was a functional B-chromosome present in *Trichomalopsis* or whether it was generated de novo in the hybridization event. Preliminary data suggests that the *Trichogramma* PSR is also of interspecific origin (van Vugt, unpublished). Other examples of the generation of B-chromosomes through interspecific hybridizations are known in plants of the genus *Coix* (Sapre & Deshpande, 1987), and has recently been shown experimentally in *Nasonia* (Perfectti & Werren, 2001).

Movement of PSR chromosomes across species boundaries is an intriguing issue with potentially broad implications. The mode of action of this chromosome, that is, the destruction of all the paternal A-chromosomes from the sperm, should allow it to cross species borders quite easily. The reason is that the original genome is 'left behind' due to the paternal genome loss induced by PSR, and the chromosome enters the new species not in hybrids which are likely to suffer fitness detriments, but in haploid males. In principle, this should permit PSR to readily cross species boundaries. How frequently this occurs naturally is unknown, but the possibility exists that PSR chromosomes in distantly related species share common origins, as opposed to independent origins.

It has been possible to transfer functional chromosomes of both *Nasonia* PSR (Dobson & Tanouye, 1998; Beukeboom & Werren, 2000) and *Trichogrammai* PSR (Stouthamer, unpublished) into closely related species by interspecific crosses. PSR appears to be limited to populations of *N. vitripennis* in the Great Basin of western North America, whereas *N. vitripennis* is found world-wide. This may suggest a very recent origin of the *Nasonia* PSR chromosome in *N. vitripennis*, perhaps from hybridization with a *Trichomalopsis* species in the same region (Beukeboom & Werren, 2000). The deserts and mountains of the Great Basin region could severely limit the spread of PSR, due to it's dependence on demes with multiple foundresses and therefore on relatively high population densities.

Considerable work in haplodiploids shows that de novo generated B-chromosomes have relatively high transmission through males (which have mitotic gametogenesis) and poor transmission through the meiotic gametogenesis of females (Ryan, Saul & Conner, 1985, 1987; Beukeboom & Werren, 1993b; Perrot-Minnot & Werren, 2001). For example de novo Bs in *Nasonia* can have 80% plus transmission through males but typically have 0–10% transmission through females. This is presumably because the de novo B lacks an effective pairing partner in meiosis. PSR chromosomes that have lost the ability to cause PGL ('non-functional') can be 'paired' as two chromosomes in females. Although cytological studies of meiosis have not been performed, genetic analysis indicates that double PSR chromosomes do not have increased meiotic transmission (Beukeboom & Werren, 1993b). Therefore, these PSR deletion chromosomes appear to be defective in regions necessary for meiotic pairing.

The meiotic instability of neo Bs has important implications for evolution of PSR like chromosomes. Any neo B would have a very short half-life within a population, due to its poor transmission through females. Therefore, such neo Bs must quickly evolve an accumulation mechanism or fitness advantage to be maintained. In the case of PSR chromosomes, this requires acquisition of the ability to cause PGL and immunity from PGL. This is an indirect argument for the 'sink' model because both steps can occur by the same mechanism. Similarly it is known that tandem repetitive DNA can undergo rapid amplification due to unequal sister chromatid exchange or other

mechanisms (Smith, 1976; Britten & Kohne, 1986). Therefore, a role for tandem repetitive DNA in PSR function is a plausible scenario based on evolutionary reasoning.

As mentioned above, we do not know whether the *Nasonia* PSR was generated by hybridization with the closely related genus *Trichomalopsis* or whether it was a functional PSR chromosome that crossed into *Nasonia* from *Trichomalopsis*. However, the two known PSR chromosomes presumably have evolved, at some point, from A-chromosomes that have undergone large deletions, thus explaining their diminutive size. There are at least two mechanisms by which this could be accomplished. One is via interspecies hybridization. A recent study by Perfectti and Werren (2001) showed instability of a chromosome that was backcrossed from one *Nasonia* species into another, resulting in spontaneous fragmentation and generation of a 'neo B' chromosome. It may generally be the case that chromosomes have higher probabilities of undergoing large deletions when introgressed into a 'foreign' genetic background. Chromosomal rearrangements could contribute to increased rates of fragmentation in interspecies hybridizations, leading to B-chromosome generation. A second common mechanism known to cause chromosomal fragmentation and 'neo B' chromosome generation is *Wolbachia*-induced cytoplasmic incompatibility. Either intraspecific or interspecific CI could therefore be a mechanism of PSR generation in haplodiploid species.

Biological control possibilities of PSR

PSR has potential as a biological control agent, although this possibility has not yet been extensively explored. The mode of action of PSR, that is, rendering eggs destined to be females into males, and the ability of the element to spread itself through the population, make it an ideal candidate for the control of pest populations. Obviously only pests with a haploid–diploid sex determination system would be suitable. In addition the PSR will only spread when the pest has a particular combination of fertilization rate and mating structure. However there are quite a large number of pests that seem to comply with these conditions. Pest species with a haploid–diploid sex determination system include whiteflies, spider mites, thrips and Hymenoptera like, yellowjackets, sawflies, seed infesting chalcids and ants.

The attraction of using PSR for pest control is that a successful introduction of PSR in pest species causes a substantial reduction in population growth, which should lead to a much lower pest population density. But how feasible is this method? For PSR to be used as a biological control agent, it must either be found naturally in a pest species or be introduced into the pest species by experimental means. If so introduced, PSR must be capable of inducing paternal genome loss, as the two PSR chromosomes do in their native species. The initial obstacle would be to enter one of the known PSR chromosomes into the pest species. Presumably this would be accomplished by microinjection of PSR-bearing sperm into eggs of the novel host species. We have no experience at this time to predict how easy this would be. However, a key feature of PSR makes this approach more likely to be successful than originally imagined – PSR normally leaves its 'host' genome behind and associates with the new genome present in the egg. Therefore, if PSR induces paternal genome loss and functions properly in mitosis in the new host, then PSR will immediately be associated with a functional genome of a haploid male from the new species. In principle, this could permit introduction of PSR into very distant host species, so long as it can function properly in mitosis and induce paternal genome loss in divergent genomic backgrounds. Several findings suggest that PSR can function in different species (Dobson & Tanouye, 1998b; Beukeboom & Werren, 2000), although the 'host range' of different PSR chromosomes have yet to be determined.

If it is discovered that PSR can be readily transferred to different haplodiploid species experimentally, then PSR also becomes a potentially useful vehicle for genetic engineering of haplodiploids. The challenge of genetic engineering in most non-model organisms is development of transformation systems. There is considerable interest in development of 'universal' transformation systems for insects using transposon based vectors that could be broadly employed. However, many challenges remain. An alternative to developing and refining transformation methods for each species would be to perform transformation in a model system and then use a second vehicle for moving the genetic modifications into the target species. PSR presents such a possibility for haplodiploids, if experiments reveal that it can be readily transferred across species boundaries. Genetic constructs could be placed on PSR in a model haplodiploid species and then the construct introduced into the target species by microinjection of sperm into eggs. Non-functional

PSR chromosomes that do not induce paternal genome loss can be readily generated by gamma ray or chemical mutagenesis (Beukeboom & Werren, 1993b), allowing introduction of the constructs into females as well as males. At this point such ideas are highly speculative, but worth contemplating for haplodiploid species.

Conclusion

PSR chromosomes are the most extreme examples of 'selfish' or 'parasitic' DNA, due to their ability to completely eliminate the genome of their 'host' while being themselves transmitted. So far, PSR chromosomes and PSR-like elements have been found in three families of parasitic Hymenoptera. PSR chromosomes are expected to evolve in haplodiploid species under a broad range of conditions where greater than 50% of the eggs in a population are fertilized (normally leading to production of female progeny in haplodiploids whereas unfertilized eggs give rise to males). Because these conditions are likely to be common in haplodiploids, we also expect PSR chromosomes to be common but generally at low frequency. PSR chromosomes appear to enter species through interspecies hybridization, and may readily cross species boundaries by these means. PSR can regulate the frequency of other sex ratio distorters under some circumstances, a may prevent the fixation of parthenogenesis-inducing bacteria in some species. However, there is currently little evidence that PSR plays an important role in shaping the sex determination mechanisms of most haplodiploids. There is potential for use of PSR chromosomes in biological control of pest insects, either directly or indirectly as a vectoring system. However, the feasibility of these approaches remains unclear. There is also considerable interest in the mechanisms by which PSR causes paternal genome loss and how these mechanisms evolve. The development of molecular approaches in haplodiploid organisms should accelerate research into these interesting questions.

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