Evolution, 46(5), 1992, pp. 1257–1268

POPULATION GENETICS OF A PARASITIC CHROMOSOME: EXPERIMENTAL ANALYSIS OF PSR IN SUBDIVIDED POPULATIONS

LEO W. BEUKEBOOM AND JOHN H. WERREN
Department of Biology, University of Rochester, Rochester, NY 14627 USA

Abstract.—*Nasonia vitripennis* is a parasitoid wasp that harbors several non-Mendelian sex-ratio distorters. These include MSR (Maternal Sex Ratio), a cytoplasmic element that causes nearly all-female families, and PSR (Paternal Sex Ratio), a supernumerary chromosome that causes all-male families. As in other hymenoptera, *N. vitripennis* has haplodiploid sex determination. Normally, unfertilized (haploid) eggs develop into males and fertilized (diploid) eggs develop into females. The PSR chromosome violates this normal pattern; it is inherited through sperm, but then causes destruction of the paternal chromosomes (except itself), thus converting diploid fertilized eggs (normally females) into haploid eggs that develop into PSR-bearing males. PSR is an extreme example of "parasitic" or "selfish" DNA. Because *N. vitripennis* has a highly subdivided population structure in nature, population-level selection may be important in determining the dynamics of PSR in natural populations. A theoretical analysis shows that subdivided population structure reduces PSR frequency, whereas high fertilization proportion (such as produced by the MSR element) increases PSR frequency. Population experiments using two deme sizes (3- and 12-foundress groups) and strains producing two fertilization proportions [wild-type (LabII)—57–67% female, and MSR (MI)—90–93% female] confirm these predictions. PSR achieved frequencies over 0.90 in 12-foundress group MSR populations in contrast to 0.20–0.40 in wild-type 12-foundress populations. PSR was selected against in wild-type populations composed of three-foundress groups. In MSR populations with three-foundress groups, presence of PSR selected against the MSR cytoplasmic element, eventually leading to low frequencies of both PSR and MSR. Complicated dynamics may occur when these two sex-ratio distorters are both present in highly subdivided populations. The existence of PSR in natural populations may depend on the presence of MSR. Results indicate that population subdivision could be important in determining the frequency of sex ratio distorters in *N. vitripennis*.

Key words.—B-chromosome, levels of selection, *Nasonia*, Paternal Sex Ratio (PSR), population structure, selfish DNA.

Received November 1, 1990. Accepted February 14, 1992.

Most species appear to harbor "parasitic" or "selfish" genetic elements, which have enhanced transmission relative to the rest of an individual's genome, but are either neutral or detrimental to the organism as a whole (Dawkins, 1976; Doolittle and Sapienza, 1980; Orgel and Crick, 1980; Werren et al., 1988). Potential examples include transposons, meiotic-drive chromosomes, non-Mendelian sex-ratio distorters, B-chromosomes, and certain cytoplasmically inherited factors. Parasitic genetic elements are relevant to fundamental questions concerning the importance of "intragenomic conflict" (Eberhard, 1980; Cosmides and Tooby, 1981) and "levels of selection" (Wade, 1978; Wilson, 1983; Maynard Smith, 1987*a*, 1987*b*; Sober, 1984, 1987; Nunney, 1985) in evolutionary processes.

By definition, parasitic genetic elements are selectively favored at the gene level. This creates a potential for intragenomic conflict within an organism. For example, in the evolution of meiotic drive, genes may be

selected to enhance or suppress drive depending upon their linkage to the driving element (Eshel, 1975; Thomson and Feldman, 1975; Charlesworth and Hartl, 1978; Wu, 1983). Intragenomic conflict also occurs between different genes affecting sex allocation, depending on their pattern of inheritance. Cytoplasmic elements are selected to produce strongly female-biased sex ratios, which are typically detrimental to transmission of autosomally inherited genes. Autosomal suppressors of such elements therefore are selectively favored (Uyenoyama and Feldman, 1978; Eberhard, 1980; Werren, 1987*b*; Taylor, 1990). Parasitic elements can also have detrimental effects on individuals and populations containing them. Therefore selection operating at different levels (i.e., gene, individual, and population) may be important in determining the frequency of these elements in nature (Werren et al., 1988).

Paternal Sex Ratio (PSR) is the most extreme example of a genetic parasite described in any species (Werren et al., 1987; Nur et al., 1988; Werren, 1991). It is a non-essential (supernumerary) chromosome that occurs in natural populations of the parasitic wasp, *Nasonia vitripennis*. As in most other hymenoptera, *Nasonia* has haplodiploid sex determination; unfertilized eggs develop into males and fertilized eggs develop into females. PSR-bearing males transmit the chromosome via sperm to fertilized eggs, but PSR then causes improper condensation and eventual loss of the paternal chromosomes, except itself (Werren et al., 1987; Nur et al., 1988). This converts diploid eggs, which would normally develop into females, into haploid eggs that develop into PSR males. PSR destroys each new set of chromosomes with which it is associated in subsequent generations.

The existence of this extreme selfish element raises several interesting evolutionary questions. One major set of questions concerns how PSR is maintained in natural populations, and how the presence of PSR affects populations carrying it. Clearly, there is the potential for group selection to act on PSR. As the chromosome increases in frequency within a population, the number of females produced declines. If PSR were to approach fixation, then the population would go extinct. It is therefore worthwhile to explore the effects of subdivided population structure on PSR frequency in controlled laboratory populations.

Nasonia vitripennis is a two to three mm parasitoid wasp that lays eggs in fly pupae, mainly blowflies and flesh flies that occur around carcasses (Whiting, 1967). Normally, 10 to 40 eggs are laid in a single pupa. Adult wasps emerge about 14 days later. The flightless males (who have vestigial wings) eclose first and then mate with emerging females within a patch of hosts. Females then disperse and search for new hosts. Due to the patchy distribution of fly pupae, natural populations of *N. vitripennis* are subdivided into temporary local mating groups (Skinner, 1983; Werren, 1983). In addition to PSR, two other non-Mendelian sex-ratio distorters occur in natural populations. Sonkiller (SK) is a maternally transmitted bacterium that prevents development of unfertilized (male) eggs laid by infected females (Skinner, 1985; Werren et al., 1986; Gherna et al., 1991). Maternal sex ratio (MSR) is a maternally transmitted cytoplasmic element that causes females to fertilize 90 to 100% of their eggs (Skinner, 1982, 1983). Females uninfected with any of these elements typically produce an increased proportion of sons with increased wasp density, in a manner generally consistent with Local Mate Competition (LMC) theory (Hamilton, 1967; Charnov, 1982; Werren, 1987*a*). There is genetic variation in sex-ratio control in natural populations (Parker and Orzack, 1985; Orzack et al., 1991).

Because *Nasonia* populations are highly subdivided in nature (Skinner, 1983; Werren, 1983), negative effects of PSR on the productivity of local populations may play a role in regulating PSR frequency. Here we present a series of experiments showing that population structure can strongly affect the frequency of PSR. In addition, we find that PSR frequency is strongly enhanced by the presence of the female-biasing element, MSR.

Theory of PSR Population Dynamics

Models for PSR population dynamics have been developed for panmictic populations (Skinner, 1987; Werren, 1987*b*) and

subdivided populations (Werren and Beukeboom, unpubl. data). Basic features of these models are presented here as a context for the population experiments to follow. The models below assume that PSR males transmit the chromosome to 100% of eggs fertilized with their sperm (and 0% of unfertilized eggs), and that PSR males are as competitive as normal males in obtaining mates. Empirical studies generally support these assumptions (Beukeboom and Werren, unpubl. data).

Panmictic Populations.—Given that x proportion of eggs are fertilized, and P proportion of females are mated to PSR males, the frequency of PSR-mated females in the next generation (P') is

$$P' = \frac{Px}{Px + (1 - x)} \tag{1}$$

The equilibrium frequency of PSR (P^*) is therefore

$$P^* = (2x - 1)/x \tag{2}$$

It follows that more than 50% of eggs must be fertilized for PSR to be maintained in a panmictic population. Because females are normally derived from fertilized eggs, a female-biased sex ratio is therefore necessary for PSR to become established. Furthermore, when most eggs are fertilized PSR can achieve appreciable frequencies. For example, if 75% of eggs are fertilized, PSR will achieve an equilibrium frequency of 0.67.

Subdivided Populations.—A detailed metapopulation model of PSR dynamics has been studied by Werren and Beukeboom (1993). The essential features of the model are presented here. Assume that hosts occur in temporary patches, where female wasps parasitize them. Mating among wasp progeny occurs in these natal patches (temporary mating groups) after the progeny emerge from their hosts. Females mate only once (or use the sperm from only one mating). Inseminated females then disperse in search of new hosts.

If N females parasitize each patch and produce x proportion of fertilized eggs, then the number of PSR-mated females produced in a particular patch will depend on the number of PSR-mated females founding that patch. The frequency of PSR-mated females produced in a patch (F_a) founded by a PSR-mated females and $N - a$ normal-mated females is

$$F_a = \left[\frac{\text{Number of PSR males produced in patchtype} - a}{\text{Number of males produced in patchtype} - a}\right] \times \left[\text{Number of females produced in patchtype} - a\right]$$

$$= \left[\frac{ax}{ax + N(1 - x)}\right][(N - a)x] \tag{3}$$

The proportion of PSR-mated females produced in the entire population in the next generation is determined by averaging over all patches in the population and dividing by the average number of females produced per patch, or

$$P' = \frac{\sum_{a=1}^{N} \text{Freq}(a)\left[\frac{ax}{ax + N(1 - x)}\right][(N - a)x]}{Nx(1 - P)} \tag{4}$$

where Freq(a) = proportion of patches in the population which have a PSR-mated foundresses and $N - a$ normal-mated foundresses. PSR will achieve equilibrium in the populations when *the average number of PSR-mated females produced per PSR-mated foundress equals the average number of females produced per foundress.*

If we assume that foundresses are distributed randomly in patches relative to whether they are PSR- or normal-mated, then Freq(a) is binomially distributed and

$$\text{Freq}(a) = \frac{N!}{a!N - a!} P^a(1 - P)^{N-a} \tag{5}$$

By iterating Equation (4), equilibrium frequencies of PSR can be determined. Figure 1 shows the equilibrium frequencies of PSR in populations with different foundress numbers (N) and different fertilization proportions (x). As can be seen in the figure, decreasing foundress number (N) reduces the equilibrium frequency of PSR, and PSR cannot be maintained in populations with a group size smaller than three foundresses.

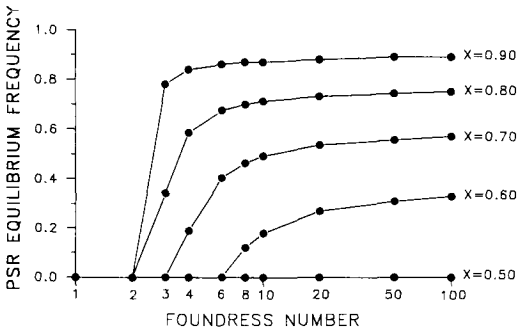

FIG. 1. Effect of subdivided population structure and fertilization proportion on equilibrium PSR frequency. Equilibrium frequencies of PSR-mated females as a function of foundress number (N) and proportion of fertilized eggs (x) are shown. Predictions are based upon a subdivided population model that assumes mating in temporary (one generation) mating groups and random dispersal of females to found new groups.

At higher foundress numbers, the equilibrium frequency is strongly influenced by the fertilization proportion. In particular, PSR can achieve much greater frequencies in populations producing a high proportion of fertilized eggs. PSR cannot be maintained in populations producing 50% or fewer fertilized eggs.

The proportion of fertilized eggs is important because PSR is transmitted via sperm and therefore only to fertilized eggs. Thus, PSR transmission increases with the proportion of eggs being fertilized. This implies that MSR may increase the transmission of PSR because MSR-cytotype females produce over 90% fertilized eggs. Group size is important because smaller group sizes result in a greater variance in PSR frequency between groups. When PSR frequency in a group is high the number of mates available to PSR males is low, and the number of males competing for these mates is high; as a consequence the number of dispersing PSR-mated females per PSR-mated foundress is relatively low. Population experiments were conducted to test predictions of the panmictic and subdivided-population models.

MATERIALS AND METHODS

Strains.—All experiments were carried out with the parasitoid wasp *Nasonia vitripennis* maintained on *Sarcophaga bullata* pupae. Two strains that fertilize different proportions of eggs were used. LabII is a standard wild-type strain; females adjust their fertilization proportion to group size in general accordance with Local Mate Competition (LMC) theory (Hamilton, 1967; Werren, 1983). LabII produces 67% females in $N = 3$ foundress situations and 57% in $N = 12$ foundress situations. In contrast, MI produces 93% females in $N = 3$ and 90% females in $N = 12$ foundress situations typical of MSR sex-ratio factors. The strain was originally collected from Macomb, Illinois, USA (Saul et al., 1965) and has been maintained in laboratory culture for over 25 years. Analysis shows that the MI sex-ratio pattern is cytoplasmically inherited.

The PSR chromosome used here is the standard strain used in previous studies (Werren and van den Assem, 1986; Nur et al., 1988). It is routinely maintained in a LabII background and was introduced into MI for the population experiments.

Population Experiments

Panmictic Population Experiment.—The experiment was conducted prior to development of molecular probes for PSR, and therefore a phenotypic assay was used. Two populations were established. A total of 5 PSR(LabII) and 95 normal LabII parasitized hosts were placed in a one gallon glass jar for population A, and 4 PSR(LabII) and 76 normal LabII hosts for population B. For this generation and each successive generation, wasp progeny were allowed to emerge and "randomly" mate within the jar to simulate a panmictic population. Then, 220 to 300 females were harvested and set individually upon single hosts for 48 to 72 hours (approximately 30 offspring develop per host). Ten to 12 days later at 25°C, progeny were in the pupal stage. Hosts were opened, the family was visually scored as either PSR (sex ratio >90% male) or normal (sex ratio <90% male) and pupae were placed within a glass jar for eclosion and mating to start the next generation. The exact sex ratios of 10 to 15 normal sex ratio families were also scored to provide an estimate of egg fertilization proportion.

Subdivided Population Experiment.—Populations using one of two strains (LabII or MI) and one of two subdivided popula-

tion structures ($N = 3$ or $N = 12$ foundress groups) were constructed, with two replicates of each. Populations are referred to as L3A, L3B, L12A, L12B, M3A, M3B, M12A, and M12B, where L and M refer to the LabII and MI strain, 3 and 12 to group size and A and B to the replicates.

Populations with group size 3 were started and maintained in 120 small vials (12 × 75 mm) with 3 females on 3 hosts, those of group size 12 were maintained in 30 large vials (23 × 95 mm) with 12 females on 12 hosts. Thus, the total number of foundresses per generation was 360 for each population. Emerging offspring were allowed to mate within the group (vial). One to two days later all vials from a population were emptied into a container from which 360 females were immediately collected (those that dispersed into a collection vial on top of the container) to start the next generation. This procedure simulated dispersal of females after mating within a group. It should be emphasized that these are temporary (one generation) groups, rather than semi-permanent demes. Generation time was 16 days at 25°C (14 days to first emergence plus 2 days for mating). Due to scarcity of hosts, the M12 population was started as one population, and split into two replicates in generation 1.

PSR chromosomes (in the same genetic background) were introduced in the L and M populations respectively at generation 0 with starting frequencies of 0.350 for the LabII populations and 0.213 ($N = 80$) for the MI populations.

Control (without PSR) populations of both strains were maintained simultaneously beginning with generation 2. The same subdivided population structures were used, but with only 10 groups of $N = 3$ and 5 groups of $N = 12$. In contrast to the experimental populations these were started anew each generation by taking mated females from a population of 30 males and 120 females that had been collected as pupae from separately maintained stock cultures. The purpose of the controlled matings was to avoid the low mating frequency sometimes observed in MI stock cultures, due to the scarcity of males. Mean fertilization proportions of these populations were used as one estimate for fertilization proportion in the experimental populations.

Population sex ratios were determined every generation by counting males and females in a sample of 200 individuals from the collection container. Total population sizes were estimated by dividing the total population weight by the weight of this sample, multiplied by 200. Population sizes were usually around 10,000 individuals, which is about 30 offspring per host. PSR frequencies of each population were determined every generation from a sample of 80 males taken from the collection container, using the squash blot assay described below.

PSR Screening Methods.—Either phenotypic or molecular assays were used in population experiments to estimate PSR frequency.

Phenotypic assay: A sample of males was removed from the population and individually mated to LabII females. Sex ratio among resulting progeny was recorded. Sex ratios of 90 to 100% male were scored as PSR. This assay can overestimate PSR frequency because some females fail to mate or mate poorly, also resulting in high sex ratios. Previous studies have found this to occur at approximately 5% (Werren and van den Assem, 1986).

Dot-blot assay: Molecular studies of the PSR chromosome have uncovered several families of tandem repetitive DNA sequences; some are specific to PSR and one is amplified on PSR but also present on the autosomes (Nur et al., 1988; Eickbush et al., 1992). Screening individual wasps for the presence of PSR was performed by DNA hybridization using a lambda clone containing repetitive DNA specific to the PSR chromosome (psr2 repeat) (Nur et al., 1988). Wasps were individually 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°C for one hour. The DNA was denatured with 1/5 volume NaOH and the solution neutralized with 1/5 volume Tris and 1/5 volume HCl. One μl of the resulting solution was spotted onto a nitrocellulose filter, which was then dried and baked at 80°C in a vacuum oven for two hours. Filters were prehy-

bridized for four hours and hybridized overnight with the PSR probe at 65°C. Prehybridization and hybridization solutions were 2× SSC, 5× Denhardt's, 1% sodium pyrophosphate, 25mM sodium phosphate, [250 mg/ml] denatured ct-DNA, and 1% SDS in distilled water. The psr2 clone was labeled by random priming (Amersham kit) with ^{32}P labeled ATP. After four rinses with decreasing concentrations of salt (4×, 2×, 1×, 0.1× SSC + 1% SDS) filters were dried and exposed to autoradiographic film for one to four days at −80°C. PSR-carrying males could easily be scored by this method, because normal males gave no signal. Known samples of PSR and non-PSR males were used to confirm the technique.

Squash-blot assay: Mass screening of many individuals in population samples was accomplished by a squash-blot method modified from Tschen et al. (1985). Wasps were squashed onto a nitrocellulose filter, which was then wetted twice on a filter paper saturated in denaturization solution (0.5 M NaOH, 1.5 M NaCl, pH13-14) for five minutes, followed by twice on a neutralization solution (1 M Tris, 1.5 M NaCl, pH8) for five minutes. Between each bath they were dried on filter paper for five minutes. After the second neutralization treatment, the wasps were removed from the filter and the filter was submerged in the neutralization solution for one minute. Filters were then dried and processed as described under the dot-blot assay.

Estimating Frequency of PSR-Mated Females (P).—For the panmictic experiment, the frequency of PSR-mated females is estimated phenotypically by scoring the frequency of females producing sex ratios greater than 90% males. For the subdivided populations, sex ratios of individual foundresses were not directly determined. Frequency of PSR-mated females was indirectly determined using the frequency of PSR among males (T_P) and the adult proportion females (f). Assuming that females mated to PSR and normal males produce equal family sizes and have equal mating success, then the frequency of PSR-mated females (P) among the parental generation is $P = T_P(1 - f)/[f + (1 - f)T_P]$. Similarly, the fertilization proportion for each generation (x) was determined using the formula $x = T_P(1 - f) + f$. These formulae are based on the observation that fertilized eggs result in either females or PSR males; they assume equal mortality of normal males, PSR males, and females.

Results

Panmictic Population Experiment

The panmictic experiment investigates the dynamics of PSR in random-mating populations. PSR was started at a low frequency of 0.05 and changes in frequency were tracked over successive generations. Two replicate populations were started.

Changes in PSR frequencies are shown in Figure 2. During the first three generations PSR rapidly increased from 0.05 to about 0.75 in both populations. From generation 3 onwards frequencies remained rather stable around 0.68 ± 0.06 SD ($N = 14$). The predicted trajectory of PSR frequency is also shown in Figure 2. To calculate the predicted, an estimate for fertilization proportion (x) is required (see Formula 2). This was determined from the sex ratios (proportion females = proportion fertilized eggs) of non-PSR families. Fertilization proportions varied between 0.70 and 0.90 over successive generations, and both populations had similar fertilization proportions. Pooling both populations, the average fertilization proportion over all generations was 0.80 ± 0.05 SD ($N = 20$). Using $x = 0.80$ in Formula 2, expected equilibrium frequency of PSR is $P^* = 0.75$. The value is significantly higher than the equilibrium PSR frequency (0.68 ± 0.06 SD) observed from generation 3 to 13.

As previously discussed, the phenotypic assay (scoring >90% male families) overestimates PSR frequency because unmated females also produce all-male progeny. Using the correction of Werren and van den Assem (1986) of $P = 1.05h - 0.053$, where h = the proportion of high male sex ratios, P = actual proportion PSR-mated females, and proportion unmated females is 0.05, the value of x becomes 0.76 and the observed equilibrium PSR frequency is 0.66 ± 0.06 SD ($N = 14$). The adjusted predicted

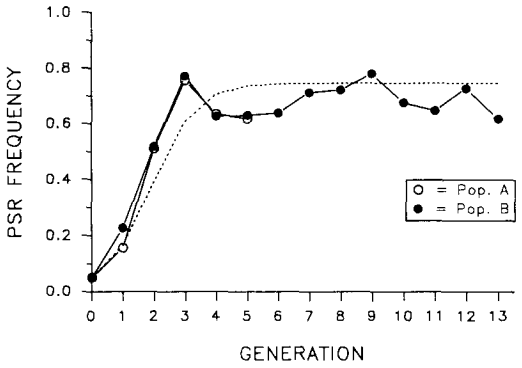

FIG. 2. Change in PSR frequency in populations A and B in the panmictic population experiment. Expected PSR frequencies from the panmictic model are shown as a dotted line.

frequency is $P^* = 0.68$. Thus, assuming a frequency of unmated females $u = 0.05$, the panmictic model is a good predictor of equilibrium PSR frequency. Unfortunately, the frequency of unmated females was not estimated in these populations.

Subdivided Population Experiment

In the second set of experiments, each population was subdivided into temporary "mating groups." Founding females laid eggs and resulting progeny mated within the demes before dispersing females were collected for the next generation. Populations were subdivided into either 3-foundress or 12-foundress demes, and were maintained for 12 generations. Strains known to produce two different fertilization proportions were used; LabII (designated L) and MI (designated M). PSR is expected to achieve high frequencies in populations with larger foundress numbers and higher fertilization proportions.

As expected, the L and M strains produced quite different fertilization proportions. Based on control populations, L3 populations produced 0.67 ± 0.06 SD versus 0.93 ± 0.06 SD for M3. L12 populations produced 0.57 ± 0.04 SD versus 0.90 ± 0.06 SD for M12. Samples are based on the average for each of 12 generations.

Figure 3 shows observed and predicted changes in frequency of PSR-mated females in these populations. Two different methods were used to predict PSR frequency. The "Fixed X Method" uses the starting frequency of PSR and average fertilization proportions from *control* populations of each strain to generate predictions, by iterating Equation (4) over successive generations. This method does not incorporate intergeneration variation in fertilization proportion

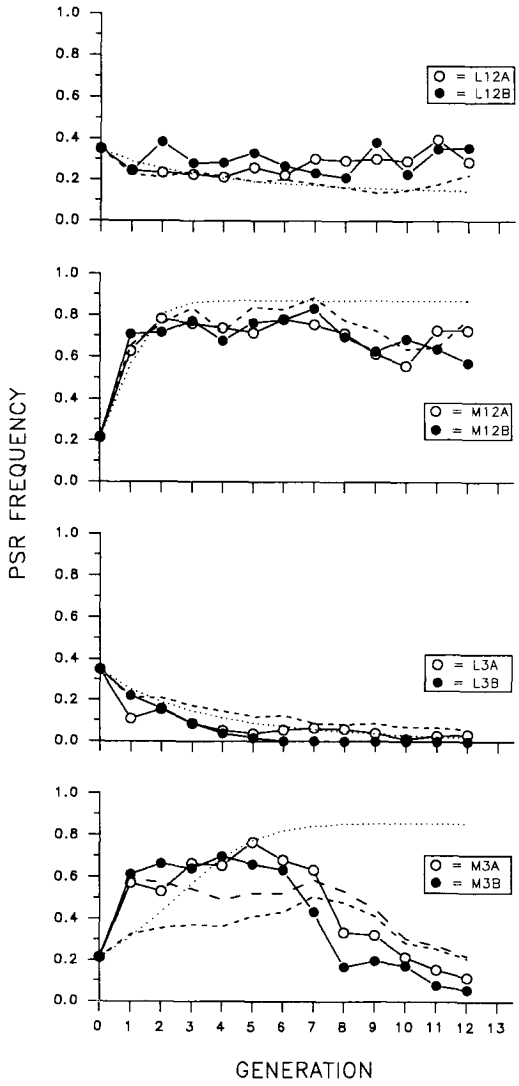

FIG. 3. Frequencies of PSR-mated females over 13 generations in experimental subdivided populations of 3 and 12 foundresses (subdivided population experiment). Data from two replicates are given for both the LabII (L) and MI (M) strain. The dotted and short dashed lines represent predicted PSR frequencies (Fixed X Method and Variable X Method, started at generation 0). The long dashed line in the M3 populations shows predictions of the Variable X Method started at generation 1 (see text).

because it uses the average fertilization proportion for each strain over 12 generations. The "Variable X Method" does incorporate such variation by using fertilization proportions each generation in Formula 4. Those fertilization proportions were estimated directly from the *experimental* populations as described in Materials and Methods, rather than from the control populations.

The two methods give similar predictions for L3, L12, and M12 populations, and generally conform to the observed changes in PSR frequency in these populations (Fig. 3). As expected, PSR was selected against in L3 populations, owing to the low fertilization proportion (L3A: $x = 0.70 \pm 0.06$ SD, and L3B: $x = 0.74 \pm 0.07$ SD) and small foundress number. PSR was lost in population L3B by generation 7, and remained at low frequency in L3A. In L12 populations, PSR was maintained in both populations at frequencies between 0.20 and 0.40. Both methods predict maintenance of PSR in these populations at frequencies between 0.10 and 0.20. Thus, observed frequencies are slightly higher than predicted.

M12 populations have a high fertilization rate, due to the cytoplasmic MSR sex-ratio distorter. PSR is therefore expected to achieve high frequencies. The Fixed X Method predicts an equilibrium around 0.85; however, PSR frequency was lower than this, although consistently above 0.60. The Variable X Method is a significantly better predictor of PSR frequency in M12 populations (Wilcoxon test; $z = 3.743$, $P = 0.0002$, $N = 24$). Recall that the Variable X Method differs from the Fixed X Method in two respects. First, fertilization proportion is estimated from the experimental populations themselves, rather than from the control populations. Second, intergenerational variation in fertilization proportion is used to estimate changes in P.

The M3 populations show the most complicated dynamics, and do not conform closely to predictions of either method (average deviation of Fixed X Method 0.37 ± 0.28 SD and Variable X Method 0.20 ± 0.09 SD, Wilcoxon test; $z = 1.929$, $P = 0.054$, $N = 24$). The basic prediction (Fixed X Method) is that PSR should rapidly increase and achieve equilibrium around 0.80. As expected, PSR does rapidly increase in M3 populations. Indeed, it achieves frequencies of 0.60 by generation 1, which was more quickly than expected. Both populations maintained frequencies around 0.65 until the sixth generation. Thereafter, contrary to expectation, PSR declined in both populations to around 0.10 by generation 12. In contrast, the Variable X Method is a poor predictor of PSR frequency *before* generation 7, but does predict the observed declining PSR frequency thereafter. This implies that the decline may have been caused by variation in the fertilization proportion.

Both predictive methods are sensitive to the starting frequency of PSR. The Variable X is a poor predictor of PSR frequency in early generations of M3, because a gradual increase was predicted whereas PSR leapt to around 0.60 in the first generation. By initializing PSR frequency at 0.60 in generation 1, it can be seen that the Variable X Method is much more consistent with observations (Wilcoxon test; $z = 2.159$, $P = 0.031$, $N = 22$, see Fig. 3). This further supports the view that the decline in PSR frequency is due to changes in fertilization proportion.

Genetic Changes in M3 Populations

The decline of PSR in M3 populations suggests that changes are occurring in these populations that select against PSR. There are three main possibilities: (1) suppressors to PSR have evolved in M3 populations, (2) the PSR chromosome has changed and has reduced transmission (or expression), or (3) the fertilization proportion has declined in M3 populations, and is no longer MSR-like.

If PSR had lost full expression, M3A and M3B PSR males mated with stock MI females are expected to produce fewer all-male families than control matings between standard PSR males and stock MI females. This was not the case; M3A and M3B PSR males from generation 13 produced 10/11 all-male families, whereas standard PSR males produced 9/9 all-male families (Fisher test: $P = 0.550$).

M3 populations were tested to determine whether an ability to suppress PSR expression had evolved. M3A and M3B females crossed to stock PSR males produced 45/45 and 47/47 all-male families, not different

from 9/9 all-male families from control crosses. If M3A and M3B females had developed a suppression ability against PSR, they were expected to produce fewer all-male families than controls mated to PSR males.

In contrast to the results above, studies do show that fertilization proportion changed in M3 populations. As can be seen in Figure 4, fertilization proportion dramatically declined over successive generations in both replicates (Spearman Rank Correlation: M3A, $r = 0.747$, $P = 0.010$; M3B, $r = 0.753$, $P = 0.009$), going from 0.93 to 0.72 in the M3A and 0.96 to 0.78 in the M3B population. By generation 13, M3A and M3B females produced fertilization proportions significantly lower than control MI females (0.84 ± 0.11 SD (N = 40) and 0.87 ± 0.06 SD (N = 40) versus 0.97 ± 0.05 SD; (Mann Whitney U-test; M3A: $z = 6.929$, $P = 4.2E\text{-}12$; M3B: $z = 6.796$, $P = 1.1E\text{-}11$), but not significantly different from control LabII females (proportion daughters 0.86 ± 0.04 SD, $N = 40$; Mann Whitney U-test: M3A: $z = 0.525$, $P = 0.600$; M3B: $z = -1.410$, $P = 0.159$). Therefore, females in these populations were producing wild-type fertilization proportions by generation 13.

M3 males did not contribute to this reduction in fertilization proportion. M3A females crossed with (non-PSR) M3A males and M3B females with (non-PSR) M3B males of generation 13 produced the same proportion of females (0.85 ± 0.04 SD, N = 24 and 0.88 ± 0.08 SD, $N = 21$ respectively) as when crossed with stock LabII males (Mann Whitney U-test; M3A: $z = -0.055$, $P = 0.956$; M3B: $z = -1.381$, $P = 0.167$).

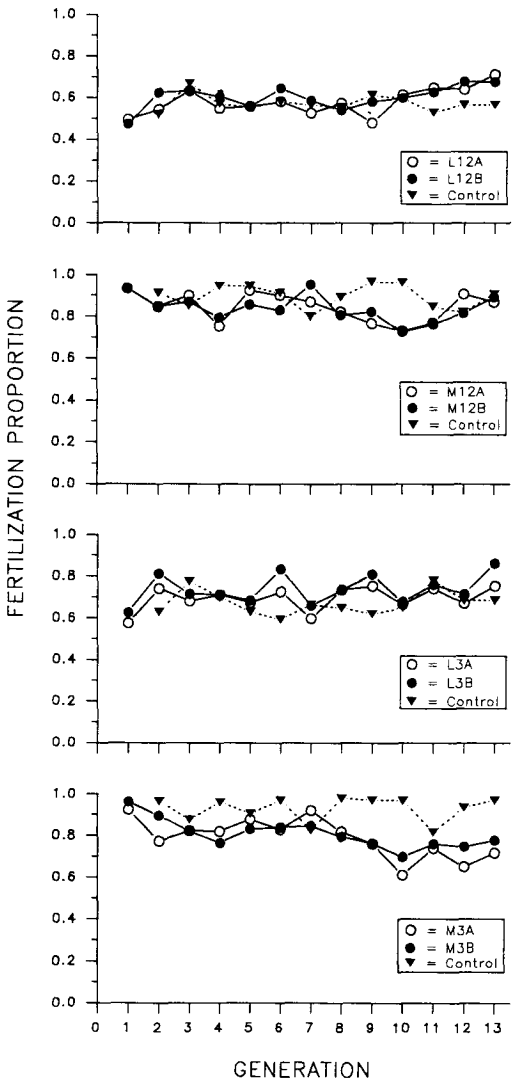

FIG. 4. Fertilization proportions of the control and experimental populations in the subdivided population experiment.

DISCUSSION

PSR is a remarkable example of selfish or parasitic DNA. It is transmitted through sperm, but then causes all-male families by destroying the paternal genome. A fundamental question concerning this chromosome is "How is PSR maintained in natural populations?" Clearly, if the chromosome were to achieve high frequency, it could drive populations extinct. On the other hand, theoretical studies (Werren, 1987*b*; Skinner 1987) indicate that PSR cannot become established in populations unless female-biased sex ratios are being produced (i.e., greater than 50% fertilized eggs). This is a consequence of haplodiploid sex determination, where fertilized eggs normally develop into females and unfertilized eggs develop into males. PSR disrupts this normal pattern, "converting" fertilized eggs into males by destroying the paternal chromosomes entering the egg with it via the sperm. Because PSR is transmitted only to fertil-

ized eggs, its frequency depends on the fertilization rate.

Evidence indicates that *Nasonia vitripennis* has a highly subdivided population structure in nature (Skinner, 1983; Werren, 1983). Population subdivision could be important in determining PSR frequency, because high frequency of PSR-mated foundresses in a local deme results in reduced numbers of PSR-mated females dispersing from the deme, due to the scarcity of females that are produced (Werren and Beukeboom, 1993).

Results of this study empirically support the view that fertilization proportion and population subdivision are important determinants of PSR frequency. In most experimental populations, PSR rapidly achieved a polymorphic equilibrium. The frequency of PSR was enhanced by high fertilization rate and decreased by population subdivision. MSR, a cytoplasmically inherited element that causes nearly all eggs to be fertilized, greatly facilitated increase of PSR. Given the number of assumptions involved in the model, it is surprising that the predictions are generally met. It was assumed that: (1) PSR transmission to fertilized eggs is 1.0, (2) PSR male mating ability is 1.0, (3) egg to adult survival of males and females is equal, and (4) females mate only once, or utilize sperm only from one mating. Various studies support these as approximate estimates (Werren and van den Assem, 1986; Beukeboom and Werren, unpubl. data), but they are not completely accurate. For example, Beukeboom and Werren (unpubl. data) show that family sizes for PSR-mated females are 10 to 20% greater than for normal-mated females. Several studies show that transmission of PSR to fertilized eggs varies between 0.9 and 1.0 (Werren and van den Assem, 1986; Beukeboom and Werren, unpubl. data). These two effects probably counterbalance each other. Furthermore, we have found that PSR males are as competitive as normal males and that multiple matings occur infrequently, at least under experimental conditions employed here.

Unexpected patterns occurred when PSR was introduced into highly subdivided (three foundress) populations composed of MSR-type females (the MI strain). In these populations, PSR increased rapidly to over 60% (as predicted), but then declined to under 10% by the 13th generation. In contrast, the population model that assumed MSR fertilization rates predicted PSR would achieve an equilibrium frequency of around 80% in these populations. Results indicate that the MSR factor had actually been severely reduced in frequency, even though the populations originally started as all-MSR. Based upon this finding, we investigated theoretically the interaction between MSR and PSR in subdivided populations. Results are presented in Werren and Beukeboom (1993). Basically, we found that presence of PSR does not select against the MSR cytoplasmic element in panmictic populations, but does select against MSR in highly subdivided (e.g., three foundress) populations. In 12-foundress populations, the model predicts that MSR frequency will be slightly reduced.

Although post hoc, the theoretical finding is qualitatively consistent with what was observed in the MI populations. However, there are clearly several unanswered questions, such as "How did the non-MSR females arise in these populations?" LabII females may have accidently been introduced; however, this must have occurred early in the experiment because both M3 replicates show the same pattern. Alternatively, some MI females may have lost the MSR sex-ratio distorter, with subsequent selection for the non-MSR type. Consistent with this latter possibility, transmission of MSR in the MI strain was found to be incomplete (Stouthamer, unpubl. data). The dynamics of MSR and PSR in highly subdivided populations may be very complex; presence of MSR greatly enhances the increase of PSR, but high frequencies of PSR tend to reduce the frequency of MSR.

Little is known about the population structure of *Nasonia* or the distribution of PSR in nature. Although it is known that *Nasonia* populations are structured into temporary local mating groups in nature (e.g., carcasses and bird nests; Skinner, 1983; Werren, 1983), the distribution of deme sizes, dispersal distances of females, and occurrence of nonmating and multiple mating are unknown for natural populations. These are likely to be important parameters in de-

termining the frequency of PSR and the other sex-ratio distorters (SK and MSR). Both chromosomal (Parker and Orzack, 1985; Orzack et al., 1991) and extrachromosomal (Werren et al., 1981; Skinner, 1985; Werren, 1991) variation for sex ratio, which occurs in *Nasonia,* will also strongly influence PSR frequency.

Spot sampling throughout North America has so far detected PSR only in Utah, Idaho, and Wyoming (Werren, unpubl. data). Intensive sampling in Utah (Skinner, 1983; Werren, unpubl. data) and New York state (Werren, unpubl. data) has found PSR at frequencies of up to 11% in Utah and 0% in New York. Local variation in PSR frequency is expected, since PSR frequency is sensitive to population structure and fertilization proportions. However, evidence to date suggests that PSR is restricted to the Great Basin, or at least Western North America. More intensive sampling is needed to resolve its actual distribution.

PSR can achieve appreciable frequencies under certain population structures, resulting in a significant reduction in the intrinsic rate of increase of such populations. Indeed, if MSR were to reach high frequencies, as is predicted theoretically (Bull, 1983; Werren, 1987*b*), then PSR should also become abundant, potentially driving the population extinct. Subdivided populations reduce PSR frequency due to local male competition and scarcity of mates. Similar mechanisms may regulate the frequency of MSR and SK, where a local scarcity of mates could give rise to virgin females, which cannot transmit these maternally inherited elements. Results presented here support the notion that subdivided population structures could be important in the dynamics of non-Mendelian sex-ratio distorters.

Acknowledgments

We appreciate the assistance of D. Eickbush, M. Riley, H. Breeuwer, M. Breeuwer-Prins, and L. Schellack in maintaining the populations and analyzing PSR frequencies. T. Eickbush and B. Burke provided use of facilities and technical advice. Comments from J. van Alphen, U. Nur, K. Reed, and two anonymous reviewers clarified the presentation of the results. This research was supported by NSF grant BSR-8796222. L.W.B. acknowledges financial support from a Fulbright Fellowship, the "Dobberke Stichting" and the "Donders Fonds."

Literature Cited

Bull, J. J. 1983. Evolution of Sex-Determining Mechanisms. Benjamin/Cummings, Menlo Park, CA USA.

Charlesworth, B., and D. L. Hartl. 1978. Population dynamics of the segregation distorter polymorphism of *Drosophila melanogaster.* Genetics 89: 171–192.

Charnov, E. L. 1982. The Theory of Sex Allocation. Princeton University Press, Princeton, NJ USA.

Cosmides, L. M., and J. Tooby. 1981. Cytoplasmic inheritance and intragenomic conflict. J. Theor. Biol. 89:83–129.

Dawkins, R. 1976. The Selfish Gene. Oxford University Press, London, UK.

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

Eberhard, W. G. 1980. Evolutionary consequences of intracellular organelle competition. Q. Rev. Biol. 55:231–249.

Eickbush, D. G., T. H. Eickbush, and J. H. Werren. 1992. Molecular characterization of repetitive DNA sequences from a B chromosome. Chromosoma. *In press.*

Eshel, I. 1975. Selection on sex ratio and the evolution of sex determination. Heredity 34:351–361.

Gherna, R., J. H. Werren, W. Weisberg, R. Cote, C. R. Woese, L. Mandelco, and R. Brenner. 1991. *Arsenophonus nasoniae,* gen. nov., sp. nov., the causative agent of the "Son-Killer" trait in the parasitic wasp *Nasonia vitripennis.* Int. J. Syst. Bact. 41:563–565.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science 156:477–488.

Maynard Smith, J. 1987*a*. How to model evolution, pp. 119–131. *In* J. Dupre (ed.), The Latest on the Best: Essays on Evolution and Optimality. MIT Press, Cambridge, MA USA.

———. 1987*b*. Reply to Sober, pp. 147–149. *In* J. Dupre (ed.), The Latest on the Best: Essays on Evolution and Optimality. MIT Press, Cambridge, MA USA.

Nunney, L. 1985. Group selection, altruism, and structured-deme models. Am. Nat. 126:212–230.

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.

Orgel, L. E., and F. H. C. Crick. 1980. Selfish DNA: The ultimate parasite. Nature 284:604–607.

Orzack, S. H., E. D. Parker, Jr., and J. Gladstone. 1991. The comparative biology of genetic variation for conditional sex ratio behavior in a parasitic wasp, *Nasonia vitripennis.* Genetics 127:583–599.

Parker, E. D., Jr., and S. H. Orzack. 1985. Genetic variation for the sex ratio in *Nasonia vitripennis.* Genetics 110:93–105.

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.

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

———. 1983. Extrachromosomal sex ratio factors in the parasitoid wasp *Nasonia* (=*Mormoniella*) *vitripennis*. Ph.D. Diss., University of Utah, Salt Lake City, USA.

———. 1985. Son-killer; a third extrachromosomal factor affecting sex ratio in the parasitoid wasp *Nasonia* (=*Mormoniella*) *vitripennis*. Genetics 109: 745–754.

———. 1987. Paternal transmission of an extrachromosomal factor in a wasp: Evolutionary implications. Heredity 59:47–53.

SOBER, E. 1984. The Nature of Selection: Evolutionary Theory in Philosophical Focus. MIT Press, Cambridge, MA USA.

———. 1987. Comments on Maynard Smith's "How to model evolution," pp. 133–146. *In* J. Dupre (ed.), The Latest on the Best: Essays on Evolution and Optimality. MIT Press, Cambridge, MA USA

TAYLOR, D. R. 1990. Evolutionary consequences of cytoplasmic sex ratio distorters. Evol. Ecol. 4:235–248.

THOMSON, G. J., AND M. W. FELDMAN. 1975. Population genetics of modifiers of meiotic drive. IV. On the evolution of sex-ratio distortion. Theor. Popul. Biol. 8:202–211.

TSCHEN, P., D. ANXOLABEHERE, D. NOUAUD, AND G. PERIQUET. 1985. Hybridization on squashed flies: A method to detect gene sequences in individual Drosophila. Anal. Biochem. 150:415–420.

UYENOYAMA, M. K., AND M. W. FELDMAN. 1978. The genetics of sex ratio distortion by cytoplasmic infection under maternal and contagious transmission: An epidemiological study. Theor. Popul. Biol. 14:471–497.

WADE, M. J. 1978. A critical review of the models of group selection. Q. Rev. Biol. 53:101–114.

WERREN, J. H. 1983. Sex ratio evolution under local mate competition in a parasitic wasp. Evolution 37: 116–124.

———. 1987*a*. Labile sex ratios in wasps and bees. Bioscience 37:498–506.

———. 1987*b*. The coevolution of autosomal and cytoplasmic sex ratio factors. J. Theor. Biol. 124: 317–334.

———. 1991. The Paternal-Sex-Ratio chromosome of *Nasonia*. Am. Nat. 137:392–402.

WERREN, J. H., AND L. W. BEUKEBOOM. 1993. Population genetics of a parasitic chromosome: Theoretical analysis of PSR in subdivided populations. Am. Nat. *In press*.

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. Tr. Ecol. Evol. 3:297–302.

WERREN, J. H., S. W. SKINNER, AND E. L. CHARNOV. 1981. Paternal inheritance of a daughterless sex ratio factor. Nature 293:467–468.

WERREN, J. H., S. W. SKINNER, AND A. HUGER. 1986. Male-killing bacteria in a parasitic wasp. Science 231:990–992.

WERREN, J. H., AND J. VAN DEN ASSEM. 1986. Experimental analysis of a paternally inherited extrachromosomal factor. Genetics 114:217–233.

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

WILSON, D. S. 1983. The group selection controversy: History and current status. Ann. Rev. Ecol. Syst. 14:159–187.

WU, C.-I. 1983. The fate of autosomal modifiers of the sex-ratio trait in *Drosophila* and other sex-linked meiotic drive systems. Theor. Popul. Biol. 24:107–120.

Corresponding Editor: B. Charlesworth