Paternal transmission of an extrachromosomal factor in a wasp: evolutionary implications

Samuel W. Skinner*

Department of Genetics, University of Wisconsin,
Madison, WI 53706, U.S.A.

Females of the haplodiploid wasp, *Nasonia vitripennis*, lay both fertilised eggs (= female) and unfertilised eggs (= male) following matings with ordinary males. Matings with males that carry the extrachromosomal *paternal-sex-ratio* (*psr*) factor result in the production of all-male broods. These male offspring are haploid and inherit only the maternal genome, yet some also inherit the *psr* factor. The proportion of males inheriting *psr* is highly correlated with the proportion of eggs fertilised in non-*psr* matings, indicating that transmission of *psr* is effected by egg fertilisation and concomitant destruction of the paternally-derived chromosomes. A simple model suggests that the frequency of the factor ought to decline rapidly in a population producing only 50 per cent fertilised eggs; this was observed.

INTRODUCTION

Chromosomal inheritance is ubiquitous among living organisms. It forms the basis of Mendel's rules and is a fundamental assumption of most of population and quantitative genetics. Extrachromosomal inheritance is nearly as ubiquitous and provides a counterpoint to chromosomal genetics. The first case was reported only a decade after the rediscovery of Mendel's rules (Correns, 1909, cited in Grun, 1976) and cases have accumulated ever since. For example, plasmids are harboured by many prokaryotes (Meynell, 1973), while mitochondria occur in virtually all eukaryotes and plastids are common in protists and plants (Margulis, 1983). Additionally, a wide variety of other elements exhibit extrachromosomal inheritance (Grun, 1976; Preer and Preer, 1977; Eberhard, 1980; Cosmides and Tooby, 1981). Often these elements have significant effects on the fitness of their carriers.

Essential to an understanding of the evolutionary genetics of extrachromosomal elements are detailed studies of their patterns of transmission for these define the basic patterns of selection and other evolutionary forces acting on them (Grun, 1976; Birky, 1978, 1983; Eberhard, 1980; Cosmides and Tooby, 1981; Birky *et al.*, 1983; Takahata,

1983). Virtually all extrachromosomal factors are maternally transmitted, although cases of biparental transmission are known (Birky, 1978). However, transmission patterns vary widely and this contrasts sharply with the restricted patterns observed for chromosomal inheritance (White, 1973). Studies of this variation may provide a deeper understanding of its genesis and maintenance in an evolutionary context, as well as shedding light on the evolution of chromosomal inheritance itself.

This paper examines the transmission process of a paternally transmitted factor, called *paternal-sex-ratio* (*psr*), which occurs in the haplodiploid wasp, *Nasonia* (= *Mormoniella*) *vitripennis*. (The factor was called 'daughterless' in Werren *et al.*, 1981). Paternal inheritance is considered a rare genetic phenomenon; moreover, in haplodiploid organisms it is paradoxical since male offspring have no fathers. This is because the haploid males arise from unfertilised eggs while fertilised eggs produce diploid females. Thus, it is of particular interest to determine the mechanism by which paternal transmission is effected.

N. vitripennis is a drosophila-sized wasp that parasitises the pupae of cyclorhaphous flies (Whiting, 1967). By controlling sperm access to eggs (King, 1962), female wasps exert behavioral control over the sex ratio among their progeny, varying it in response to several environmental cues

* Present address: Department of Biology, Indiana University, Bloomington, IN 47405, U.S.A.

(reviewed in Charnov, 1982). In particular, ordinary wasps produce very female-biased sex ratios when ovipositing in isolation but lower the sex ratio in the presence of other females. The variation is due to alterations of the primary (zygotic) sex ratio rather than changes in the secondary sex ratio resulting from differential survival of the sexes. Genetic variation for this behavior is known (Parker and Orzack, 1985). In addition four extrachromosomal factors are known to affect the sex ratio in certain wasp strains (Ryan and Saul, 1968; Werren *et al.*, 1981; Skinner, 1982; 1983; 1985). Three of the four occur at significant frequencies in natural populations of the wasp (Skinner, 1983).

The *psr* factor is paternally inherited and causes the production of all-male broods (Werren *et al.*, 1981; Skinner, submitted). (A brood consists of the progeny of a single mating.) The males are haploid and, by the use of genetic markers, have been shown to inherit only maternally-derived genes. However, some but not all of the male offspring also inherit *psr*. The sizes of *psr* broods and non-*psr* broods are equivalent, indicating that the production of all-male broods is not due to mortality of the female offspring. Since *psr*-males produce viable sperm that are transmitted to the female in copulation, I hypothesised that fertilisation of eggs is involved in transmission of the *psr* trait. However, the hypothesis requires that the paternally-derived chromosomes be destroyed before or after fertilisation. Thus what would have been a diploid female is converted into a haploid male that inherits the *psr* factor. The hypothesis predicts that the proportion of males inheriting the trait in a brood ought to correspond to the proportion of eggs fertilised; the variable sex ratio behavior of the wasp allows this to be tested experimentally. This paper presents evidence consistent with the hypothesis and, by means of a simple model, examines the resulting evolutionary consequences. Also presented are the results of a population experiment which conforms to the model. Werren and van den Assem (in press) have also examined *psr* transmission.

MATERIALS AND METHODS

Five strains of the wasp were used for the experiments. Names and relevant details concerning these strains are as follows:

psr-3: A wild-caught strain that was used in all experiments; it was maintained each generation by crossing males from all-male broods to females of the ScDr strain.

ScDr, *cb*⁺: Common lab strains that are free of extrachromosomal factors known to affect the sex ratio. The ScDr strain is derived from *cb*⁺ and is homozygous for a scarlet-eyed allele at the R-locus (Whiting, 1967); *cb*⁺ carries the wild-type allele at the locus. Both exhibit statistically equivalent sex ratio behavior (Skinner, 1983).

TC: A wild-caught strain that is infected with the *maternal-sex-ratio* (*msr*) factor. The factor induces females to produce a higher proportion of daughters than do the ScDr and *cb*⁺ strains (Skinner, 1982).

211A: A strain of ScDr females which was artificially infected with the *son-killer* bacterium (Skinner, 1985). The bacterium kills unfertilised (male) but not fertilised (female) eggs (Skinner, unpublished).

Details of rearing the wasp and general lab protocols are given in Skinner (1985). In all but the population experiment (E), females were given 24 hours to parasitise hosts. Hosts were pupae of the fleshfly *Sarcophaga bullata* which were obtained from Grubco, Inc (Hamilton, OH). All experiments were conducted under constant light at 23°C.

Five separate experiments were conducted. In three of these (Expts. A-C), a correlation was sought between the proportion of fertilised eggs and the proportion of males inheriting the *psr* factor. All three experiments employed the same general protocol. For control crosses, non-*psr* males were mated to females, yielding broods with both sexes. The proportion of daughters in these broods (*i.e.*, the sex ratio) represents the proportion of eggs fertilised; this will be referred to as the "fertilisation rate". For experimental crosses, *psr* males (parental gen.) were mated to equivalent females which then oviposited under equivalent circumstances. These matings resulted in the production of all-male broods (with occasional females, see Skinner, submitted). In order to estimate the transmission rate of the *psr* factor, males from these broods (*i.e.*, the F₁ progeny) were subsequently tested for inheritance of *psr* by mating them to females and scoring the resulting broods as all-male (*psr*) or mixed-sex (non-*psr*). The proportion of males siring all-male broods is the proportion of males inheriting the trait in the original cross. In order to control for genotypic effects, control and experimental males had the same nuclear genotype (ScDr) for each experiment.

One aspect of the above procedure warrants further elaboration. Since *psr*-males are distinguished from non-*psr* males only by the production

of all-male broods, males only can be scored retrospectively. For all three experiments, the parental generation of males were drawn from all-male broods in the *psr*-3 stock and scored as *psr* if they in turn sired all-male broods. The parental males that sired mixed sex broods are not included in table 1 as they were considered non-*psr*. Extensive analysis of over 100 of these putative non-*psr* males has shown this to be appropriate (Skinner, submitted). Conversely, because unmated females also produce all-male broods, the use of an all-male progeny to score a male as *psr* may be incorrect. To reduce this source of error, it was necessary to observe every mating to ensure insemination and to do control matings involving males from non-*psr* strains. Of 628 control matings only 4 (0.64 per cent) resulted in an all-male brood. Thus the use of an all-male progeny to score a male for the *psr* factor is actually quite reliable. (Rarely a *psr* brood contains one or a few females, yielding a sex ratio between 1 per cent and 20 per cent daughters; these broods were carefully retested to demonstrate the presence of *psr*. This phenomenon is extensively analysed in Skinner, submitted).

The fourth experiment (D), involving the son-killer bacterium, utilised the same protocol as above, however, the relevant data consist of the brood sizes produced in the control and experimental matings. Additionally, a set of virgin females were given hosts and the brood sizes were recorded. There is no inhibition of oviposition in virgin wasps (Whiting, 1967).

The fifth experiment (E) examined the population dynamics of *psr* in a panmictic population. An experimental *psr* stock was established by mat-

ing 30 males from *psr*-3 broods to 30 ScDr females. Each female was then given a host to parasitise in isolation before they were combined in a stock vial (a drosophila shell vial) with 10 cc of hosts (approximately 40–50 hosts). The females were given 48 hours to parasitise these hosts before being removed and discarded. The first set of hosts (isolation) provides a means of estimating the initial frequency of *psr*; the second (group) initiates the experimental population. The estimate of *psr* frequency in the first set of hosts was confirmed by subsequent testing of the males from each brood. A control stock was established in the same fashion but using non-*psr* ScDr males.

Both stocks were followed for four subsequent generations, with each generation initiated by 30 females that were randomly drawn after most of the wasps in the original vial had emerged from their hosts and mated. In each generation, all wasps in a vial were sorted by sex and counted after death. At the end of the five generations, males from the experimental stock were individually assayed for *psr* by making crosses to ScDr strain females and scoring the all-male broods as *psr*.

Statistics: Average brood sizes from each experiment are given as: mean $\pm$ standard error (n = the number of ovipositing females). 95 per cent confidence limits for the estimates of the fertilisation and transmission rates (table 1) are taken from Rohlf and Sokal (1969, table W). The χ^2 statistic is used to test for differences among rates; this is justified because the fertilisation and transmission rates are approximately binomially distributed.

Table 1 Transmission of the *psr* factor under different fertilisation regimes. Fertilisation rates are estimated from the crosses involving non-*psr* males; transmission rates refer to the crosses involving *psr*-males. 95 per cent confidence intervals are provided for each estimated rate (Rohlf and Sokal, 1969, Table W)

Expt.	Matings $\delta \times \varphi$	Oviposition Context (φ : host)	N	Offspring # δ # φ		Non- <i>psr</i> Males	<i>psr</i> Males	Fert. rate or Trans. rate
A	non- <i>psr</i> $\times$ ScDr	1:1	56	323	1710			0.84 (0.81–0.86)
	<i>psr</i> $\times$ ScDr	1:1	100			38	158	0.81 (0.75–0.86)
B	non- <i>psr</i> $\times$ cb ⁺	2:1	21	281	431			0.61 (0.56–0.65)
	<i>psr</i> $\times$ cb ⁺	2:1	24			67	110	0.62 (0.54–0.69)
C	non- <i>psr</i> $\times$ TC	1:1	108	145	3122			0.96 (0.94–0.97)
	<i>psr</i> $\times$ TC	1:1	37			15	244	0.94 (0.89–0.97)

RESULTS

Transmission of psr between generations

Experiments A-C utilised the variable sex ratio behavior (fertilisation rate) of the wasp to determine the correlation between fertilisation of eggs and transmission of *psr*.

The first experiment (A, table 1) examined the situation of a single female ovipositing in isolation. For the control cross, females of a common lab strain (ScDr; see methods) were mated to non-*psr* males to obtain an estimate of the fertilisation rate. Fifty-six mated females produced progeny when given a single host in isolation for 24 hours. Of the 2033 progeny, 84 per cent were females. In the experimental cross involving *psr* males, 100 all-male broods were obtained. On testing a subset of males from these broods, 158 (out of 196 successfully tested) had inherited the *psr* trait— a transmission rate of 81 per cent. The fertilisation rate in the controls and the transmission rate in the experimentals do not differ significantly by a χ^2 test ($\chi^2 = 1.61$, $df = 1$, n.s.). Mean brood sizes were equivalent in the two crosses (control: 34.3 ± 1.40 , $n = 56$; experimental: 36.6 ± 0.95 , $n = 100$).

Conditions in Experiment B were manipulated so that females would reduce the proportion of fertilised eggs that they laid. This should lead to a reduction in the proportion of males inheriting the *psr* factor. Females (*cb*⁺ strain) were mated and then, during oviposition, each female was paired with an ScDr female. (The ScDr strain was derived from the *cb*⁺ strain but differs in possessing a mutant eye-color allele, so that the progeny of the two strains can be distinguished.) The fertilisation rate equaled 0.61 in the controls (B, table 1) while the transmission rate equaled 0.62 in the experimental cross. As anticipated, both rates were significantly lower than in Experiment A (by χ^2 tests) but are not themselves significantly different. There were also no significant differences in mean brood size (control: 27.8 ± 3.42 , $n = 21$; experimental: 24.4 ± 1.82 , $n = 24$).

Experiments A and B utilised females that are free of the known extrachromosomal sex-ratio factors. Experiment C employed females with one of these factors in order to increase the fertilisation rate. Females were taken from a strain (TC) that is infected with the *maternal-sex-ratio* (*msr*) trait. *msr* is maternally inherited and causes isolated females to produce a higher proportion of fertilised eggs than do non-*msr* females (Skinner, 1982). In the control crosses, 96 per cent of the eggs were fertilised (C, table 1). In the cross of *psr* males to

msr females, all-male broods were produced. Thus, in some sense, *psr* is dominant over *msr*. On testing the male offspring, 94 per cent of them were found to have inherited the *psr* trait. The two rates do not differ significantly; however, both are significantly higher than in Experiments A and B. Again, there were no significant differences in brood size (control: 33.8 ± 0.89 , $n = 108$; experimental: 31.7 ± 1.28 , $n = 37$).

In each experiment there is a very good correspondence between the proportion of eggs fertilised and the proportion of males inheriting the *psr* trait. This is strong circumstantial evidence in support of the fertilisation-transmission hypothesis. Evidence of a different sort may be adduced from one additional experiment (D). Here females were used that were infected with a second maternally-transmitted trait, *son-killer* (*sk*). This trait is due to a bacterium (Huger *et al.*, 1985; Werren *et al.*, 1986) that kills approximately 80 per cent of all unfertilised eggs but does not affect fertilised eggs (Skinner, unpublished). Thus, one observes large differences in the numbers of surviving offspring from virgin and mated females that are infected with the bacterium. Although both types lay equal numbers of eggs (Skinner, 1985), there is low survivorship among the progeny of virgins because all of their eggs are unfertilised. If *psr* is transmitted via fertilisation, one expects the numbers of surviving offspring to be equivalent between infected females mated to non-*psr* and infected females mated to *psr* males.

In control crosses with non-*psr* males, a strain of infected females (211A) produced an average of 20.4 ± 1.33 ($n = 29$) viable offspring which were almost entirely female while those mated to *psr* males produced 19.6 ± 1.08 ($n = 36$) viable sons. By contrast, virgin *sk*-infected females produced only 5.1 ± 0.60 viable sons ($n = 27$). This suggests that a proportion of the males in the *psr* cross are being rescued from the lethal effects of the bacterium, exactly as anticipated if *psr* is inherited from fertilized eggs.

Population dynamics of psr

Each of several initial attempts to maintain *psr* by routine stock maintenance procedures quickly resulted in loss of *psr* from the stock. Indeed, the original *psr* strain of Werren *et al.* (1981) was lost in this fashion. After new *psr* strains were collected and reliable maintenance procedures had been worked out, an attempt was made to follow the loss of *psr* under similar conditions. The results of this experiment (E) are presented in table 2.

Table 2 Decline in the frequency of *psr* in a random mating experimental population as assayed by changes in the population sex ratio. Expected population sex ratios are calculated based on the model presented in the text [expected SR = $(1-p)f$]. The control population assays the sex ratios produced by females in the absence of *psr* and provides the estimate for f in the model

Gen.	Sex ratios of Control population			Sex ratios of Experimental population			Expected Sex ratio
	# ♂	# ♀	% ♀	# ♂	# ♀	% ♀	% ♀
0	846	779	48	2359	333	12	17
1	495	528	52	787	348	31	30
2	539	694	56	830	827	50	36
3	777	689	47	540	353	40	39
4	661	667	50	1300	923	42	41

Across the 5 generations of the experiment, the control stock produced a population sex ratio of approximately 50 per cent females, as expected (Charnov, 1982). For the experimental stock, the initial frequency of *psr* was estimated at 0.67 and this resulted in a population sex ratio of 12 per cent. Population sex ratios increased over the subsequent generations as expected if *psr* is decreasing in frequency. At the end of the five generations, 137 males from the experimental stock were successfully assayed for *psr*. Only 22 sired all-male broods yielding an observed frequency of *psr* of 0.16 with a 95 per cent confidence interval of 0.095 to 0.247.

DISCUSSION

All the evidence from the preceding experiments supports the hypothesis that transmission of *psr* is effected by fertilisation of eggs. Werren and Assem (in press) also present evidence for a relationship between fertilisation of eggs and transmission of *psr*. Yet if the hypothesis is correct, it necessarily implies that the paternal set of chromosomes is destroyed or lost before or shortly after fertilisation, because the males that inherit the *psr* factor are haploid and have been shown to inherit only the maternal genome (Skinner, submitted).

The mechanisms by which the paternal chromosomes might be selectively destroyed or lost are wholly unknown. However, the phenomenon is not without precedent. Cytoplasmic incompatibility in *Nasonia* is already known to involve selective destruction of paternally transmitted chromosomes after fertilisation but just prior to syngamy of the male and female pronuclei (Ryan and Saul, 1968). Also, selective loss of paternally transmitted chromosomes is known in other

insects (Brown and Chandra, 1977). Examination of spermatogenesis in *psr* males by light and electron microscopy has revealed no unusual phenomena and mature sperm readily stain for DNA (Werren *et al.*, 1981; Skinner, submitted). Although negative evidence, it suggests that the loss of the sperm-derived chromosomes may occur after fertilisation and hence could involve differential regulation of the maternally and paternally derived genomes. Evidence at the molecular level for differences between maternally and paternally derived chromosomes has recently been obtained in birds (Groudine and Conkin, 1985). The molecular basis for these phenomena might provide clues to the regulation of the very earliest stages of development.

The observation that transmission of the *psr* factor varies with the proportion of eggs being fertilised has significant implications for the population genetics of the trait. This may be illustrated by a simple model which assumes that there are two types of males (*psr* and non-*psr*) in a large panmictic population. These occur with frequencies p and $1-p$, respectively. The parameter, f , is the proportion of eggs fertilised in a mating; alternatively, f can represent the average fertilisation rate in the population at large. With these assumptions, a fraction pf of the progeny are *psr* sons from matings with *psr* males, $p(1-f)$ are non-*psr* sons from such matings; $(1-p)(1-f)$ are normal sons from matings with non-*psr* males and $(1-p)f$ are daughters from these matings. Females produce equivalent numbers of offspring regardless of the type of male they mate with. Thus the proportion, p' , of *psr* males in the next generation is given by

$$p' = pf/[pf + p(1-f) + (1-p)(1-f)] \\ = pf/[p + (1-p)(1-f)].$$

If $f > 1/2$, there is a stable, globally attracting, equilibrium at $\hat{p} = (2f-1)/f$. Thus the model predicts that in a panmictic population where the expected sex ratio is 50 per cent females (Fisher, 1958; Charnov, 1982) the *psr* factor will be lost. This result is quite surprising because an extra-chromosomal factor causing uni-sexual broods is normally expected to spread to fixation in a population (Lewis, 1941; Cosmides and Tooby, 1981).

The reason for this anomalous behavior apparently lies in the production of a complement of non-*psr* sons in the matings with *psr* males. This represents a form of incomplete transmission. To illustrate the effect of these non-*psr* sons, imagine a situation where a mating with a *psr* male results in the production of f *psr* sons but no viable non-*psr* sons. Here the equation for between generation change in *psr* frequency is $\Delta p = p(1-p)(f-0.5)/z$ which yields no polymorphic equilibria [$z = 0.5 < pf + (1-p)(1-f) > 0.5$]. As illustrated in fig. 1 (line b), when no competing brothers are produced, the factor is fixed whenever f is greater than 0.5.

Figure 1 Equilibrium frequency of the *psr* factor in a panmictic population. The equilibrium frequency ($\hat{p}$) varies as a function of the fertilisation rate of ovipositing females. (a) Population in which there is no mortality of the males arising from unfertilised eggs. (b) Case in which matings with *psr* males result in the production of f *psr* sons only. See text for details.

Not only are the population genetics of *psr* anomalous, but the changes in frequency are remarkably fast when the frequency is away from equilibrium. As illustrated in fig. 2, dramatic changes in frequency are predicted to occur in only a few generations. These theoretical expectations are highly congruent with the observed changes in Experiment E and in Werren *et al.* (1981). For experiment E, *psr* was introduced at a high frequency ($p = 0.67$) into a population with a fertilisation rate of 0.5 (*viz.*, the control population)

Figure 2 Dynamics of the *psr* factor in panmictic populations. With a 50:50 sex ratio ($f = 0.5$), *psr* rapidly declines in frequency, going from 0.8 to 0.16 in five generations and eventually being lost. With a higher sex ratio of 80 per cent females ($f = 0.8$), the factor rapidly increases to its equilibrium value of 0.75 from an initial frequency of 0.05.

and within 4 generations fell to a frequency of $p = 0.16$. This is in substantial agreement with the value of $p = 0.18$ predicted by the model. Moreover, the generation by generation frequency of the factor was indirectly monitored by observing the population sex ratio. From table 2 it is clear that the agreement between the observed and the expected sex ratio was remarkably close for each of the five generations. Conversely, Werren *et al.* (1981) observed that when *psr* was introduced at a low frequency (5 per cent) into a population of females producing approximately 80 per cent fertilised eggs (daughters) the factor increased to a frequency of 63 per cent within two generations and maintained that approximate frequency for several more generations. Here the predicted frequency was 75 per cent.

The frequency of the *psr* factor in natural populations of the wasp was estimated at approximately 12 per cent (Skinner, 1983). What maintains the factor at this frequency? No definitive answer is possible at present, however, two aspects of the wasp's biology are likely to be relevant. Firstly, the observation that *psr* is "dominant" over both *msr* and *sk* is significant, for the presence of either one or both of these factors in a natural population leads to an increase in the average fertilization rate (f). Thus the presence of either factor (together they infect 25-30 per cent of all wild-caught females) facilitates the spread and maintenance of *psr*. (Curiously, *psr* can be shown to have no adverse effect on the spread of either of the other factors since it affects the progeny of infected and uninfected females equally.) Secondly, the demic

population structure of *Nasonia* also is likely to facilitate maintenance of *psr* because all types of females produce higher proportions of fertilised eggs in subdivided populations (Skinner, 1983). (The above model is not correct for strongly subdivided populations but may be approximately correct for moderate subdivision.)

Both the preceding experiments and theoretical models reveal the transmission dynamics of *psr* to be governed by the sex ratio behavior of females and the presence of additional extrachromosomal factors affecting the sex ratio. Careful disentangling of these phenomena through genetic, behavioral and ecological studies should yield both mechanistic and evolutionary insights concerning the nature of extrachromosomal inheritance.

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