SON-KILLER: A THIRD EXTRACHROMOSOMAL FACTOR AFFECTING THE SEX RATIO IN THE PARASITOID WASP, *NASONIA* (=*MORMONIELLA*) *VITRIPENNIS*

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ABSTRACT

An extrachromosomal factor, termed son-killer (*sk*), affects the sex ratio in a parasitoid wasp, *Nasonia* (=*Mormoniella*) *vitripennis*. The factor is maternally transmitted and alters the secondary sex ratio of an infected female through mortality of approximately 80% of the male embryos. No effect on the primary (zygotic) sex ratio is observed. Ninety-five percent of the daughters of an infected female inherit son-killer. The factor can also be transmitted contagiously when the progeny of infected and uninfected females develop simultaneously on a single host. In newly infected strains, the sex ratio effects are equivalent to those in the original.

EXTRACHROMOSOMAL factors that influence patterns of sex allocation (*sensu* CHARNOV 1982) are known or suspected in a variety of organisms. There are two basic types. "Sex-converting" factors convert one sex into the other, and "sex-killing" factors kill one sex but not the other.

In at least a dozen species of insects, skewed sex ratios are caused by extrachromosomal factors of the sex-killing type (see UYENOYAMA and FELDMAN 1978 for review). These sex ratio phenomena are best understood in Drosophila in which two or more types are known (WILLIAMSON and POULSON 1979), but they also are reported in other Diptera (ANDREADIS and HALL 1979 and references therein), Lepidoptera (EARLE and MACFARLANE 1968; CLARKE, SHEPPARD and SCALI 1975), Coleoptera (SHULL 1948; LANIER and OLIVER 1966) and Hemiptera (LESLIE 1984). In every case, the factors are maternally inherited and cause mortality of the male offspring.

Extrachromosomal sex ratio factors are also known in the hymenopteran, *Nasonia* (=*Mormoniella*) *vitripennis*. Nasonia is a small gregarious wasp that parasitizes the pupae of cyclorrhaphous flies, especially those in the families Calliphoridae and Sarcophagidae (WHITING 1967). WYLIE (1976 and earlier), HOLMES (1970, 1972), WERREN (1980, 1983) and others have demonstrated that Nasonia females vary the sex ratio among their progeny as a function of the context in which they oviposit (reviewed by CHARNOV 1982). For example, isolated females produce very female-biased ratios, but, when ovipositing in groups, the sex ratios of individual females shift gradually toward 50:50 with

increasing group size (WERREN 1983). These shifts are in the primary (zygotic) sex ratio. The proximate basis for this sex ratio control lies in the hymenopteran system of haplodiploidy: fertilized eggs become females; unfertilized eggs become males. Females determine the sex ratio by controlling sperm access to eggs (see KING 1962; GERBER and KLOSTERMEYER 1970; COLE 1981).

In the course of fieldwork on Nasonia, two extrachromosomal factors affecting the sex ratio have been discovered. WERREN, SKINNER and CHARNOV (1981) reported a paternally inherited factor, termed paternal sex ratio (*psr*), that causes the production of all-male broods. (Note: *psr* was originally called "daughterless.") SKINNER (1982) reported a maternally inherited factor, maternal sex ratio (*msr*), that skews the sex ratio toward females. In neither case is the sex ratio effect due to mortality of the missing sex. Thus, both are of the sex-converting type and, hence, differ in mechanism from those previously reported in insects.

This paper reports the discovery of a *third* extrachromosomal factor affecting the sex ratio in Nasonia; it, too, has been recovered from natural populations of the wasp. The factor, termed son-killer (*sk*), is similar to those discovered in other insects since it alters the sex ratio through mortality of male offspring. The experiments reported here (1) compare the distributions of sex ratios obtained from *sk*-infected females with those of uninfected females, (2) estimate the average mortality among the sons of *sk*-infected females and (3) demonstrate that *sk* is both maternally and contagiously transmitted.

MATERIALS AND METHODS

General: All experiments employed *Sarcophaga bullata* as hosts; these were reared in the laboratory on beef liver. Hosts were refrigerated at 7° until use. Wasps for experiments were drawn from three laboratory strains maintained by allowing 15 inseminated females from each generation to freely parasitize 40–50 pupae until the females died. The cb^+ and ScDr strains were obtained from Carolina Biological Supply Company in 1978. cb^+ is wild type (brown) for eye color, whereas the ScDr strain is homozygous at the *R* locus for an eye color allele ($R^{st\text{-}DR}$) that has a distinctive scarlet phenotype (SAUL, SAUL and BECKER 1967; WHITING 1967). Both are free of all extrachromosomal sex ratio factors. The *sk*-infected strain utilized here (HEB-3) originated from a female trapped near Heber, Utah, in July 1982. Only the wild-type eye color allele is present in this strain. All three strains are highly isogenic due to periodic passage through population bottlenecks. This was done to provide a genetically homogeneous background on which to observe the effects of the son-killer factor.

All experiments were carried out under constant light at 23° ± 1°. Development from egg to egg-laying adult wasp takes approximately 3 wk under these conditions. For experiments, wasps were isolated as first- or second-day "black" pupae; eye colors and sexes are easily distinguished at this stage. The sexes differ in genitalia, relative size of the wing pads and color of the head and antennae.

Two types of mating scheme were used. In *mass-matings*, male and female pupae were placed together in a two female to one male ratio. When approximately three of four had eclosed, they were given honey to feed on and the remaining pupae were removed. Forty-eight hours later, females were given hosts to parasitize. For *pair-mating*, males and females were isolated as pupae, the sexes being kept separate. Twenty-four hours after the wasps had eclosed, each female was paired with a male and observed until she mated (usually <1 min). Males were removed after an interval of 6 hr, and each female was given a drop of honey to feed on. Eighteen hours later, the females were given hosts. Under both procedures, females were approximately 48–60 hr posteclosion at the time they received hosts. Regardless of the pretreatment, single females were always isolated with one host to parasitize for 24 hr in a 12 × 75-mm test tube. Offspring were counted and sexed 15–16 days later.

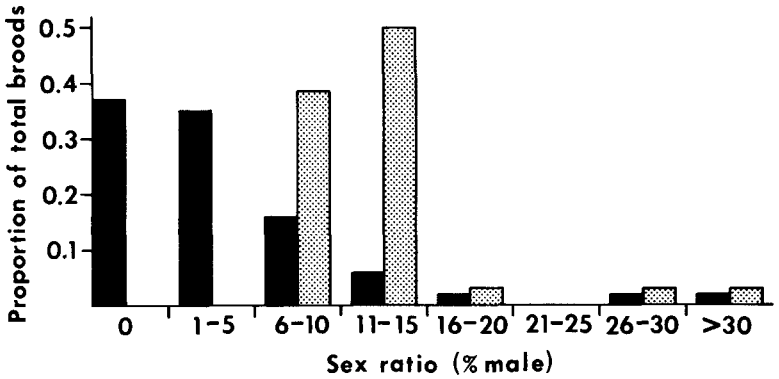

FIGURE 1.—Distribution of sex ratios obtained from *sk*-infected wasps of the HEB-3 strain (solid bars, $N = 46$) and of uninfected wasps of the ScDr strain (stippled bars, $N = 28$). Females were pair mated to males of their own strain and then were given a single host to parasitize for 24 hr.

Sex ratio distributions are compared using the Kruskal-Wallis one-way analysis of variance. Means are given with standard deviations rather than standard errors because of non-normal distributions. Comparisons between means are made with Student's *t*-test when variances are equal; otherwise, a modified form is used (SOKAL and ROHLF 1969, p. 374).

Assaying male mortality: To assay male mortality, virgin females were used, in order to take advantage of the fact that they produce only male (haploid) progeny. There is no inhibition of oviposition in virgins (WHITING 1967). To assess a mortality rate accurately, both living and dead individuals should be counted. However, due to the difficulty of dissecting the host puparium from the host pupa without damaging the latter (resulting in covering everything with host hemolymph), in only one case (experiment 2) was this attempted. For the other experiments, only the inviable (unhatched) eggs or only the surviving pupae were counted.

When counts of unhatched eggs were to be made, host pupae were partially buried in fine sand, exposing only one end of the puparium for parasitization. A test tube containing the virgin female was then inverted over the pupa. This localized the female's eggs enabling them to be counted easily. This procedure has no detectable effect on a female's brood size (S. W. SKINNER, unpublished results); in fact, in the field, hosts frequently are partially buried in the soil. At 23°, eggs hatch in approximately 36 hr; to ensure that hatching had been completed, hosts were opened and the unhatched eggs were counted destructively 48–60 hr after removing the parasitizing female. Hosts that were unparasitized or damaged on opening were discarded, as were the infrequent hosts on which fewer than 20 eggs were laid. The latter procedure allows more certain discrimination between infected and uninfected females.

RESULTS

Experiment 1: sex ratio distributions from sk*-infected and uninfected females*: Females were isolated from the HEB-3 (*sk*-infected) and ScDr (uninfected) strains. Each female was pair mated to a male from the same strain before being given a host for parasitization (one host per female for 24 hr). Figure 1 compares the distribution of sex ratios obtained from the *sk*-infected and uninfected wasps. (Three HEB-3 females that were not *sk* infected, using the son-killer assay of experiments 2 to 4, are excluded from the data.) Note that both types produce female-biased sex ratios, but, although uninfected wasps produce the typical sex ratio of 11 to 15% males (SKINNER 1982; WERREN 1983), *sk*-infected wasps produce even more biased sex ratios of 0 to 5% sons. The two distributions are significantly different ($H = 25.27$, d.f. $= 1$, $N = 71$, $P \ll 0.001$, two-tailed).

TABLE 1

Offspring numbers from mated females with and without the son-killer factor

Experiment	Strain	*sk*	*N*	Females (mean)	Males (mean)	Estimated mortality (%)
1	HEB-3	+	43	42.3 ± 14.21 (a)	2.1 ± 4.01* (b)	
	ScDr	−	28	44.4 ± 16.89 (c)	8.6 ± 14.84 (d)	74
5a	ScDr	+	58	26.0 ± 7.33* (a)	1.0 ± 1.20*** (b)	
	ScDr	−	45	29.2 ± 8.49 (c)	4.8 ± 2.97 (d)	77
5b	cb^+	+	45	43.7 ± 18.76 (a)	0.8 ± 1.08*** (b)	
	cb^+	−	39	42.9 ± 15.45 (c)	7.3 ± 7.41 (d)	89

Estimated male mortality is calculated as 1 − (bc/ad). This assumes equivalent primary sex ratios and equivalent female mortality in the *sk*-infected and uninfected clutches. Comparisons are between uninfected clutches and the preceeding *sk*-infected clutches within a sex. Where variances are unequal a modified *t*-test is used (see SOKAL and ROHLF 1969, p. 374).

* $P < 0.05$; *** $P < 0.001$.

A few females in both strains produce relatively high sex ratios (*i.e.*, >25% sons). This is common (*e.g.*, SKINNER 1982) and presumably represents random variation. It is unlikely to be due to the production of diploid males as occurs in some other Hymenoptera (WHITING 1967). When single (uninfected) females are given a series of hosts, they produce both low and high sex ratios, resulting in distributions similar to those of the uninfected females in Figure 1. Moreover, males from high sex ratio broods sire daughters of normal fecundity (S. W. SKINNER, unpublished results). Diploid males produce diploid sperm and, hence, triploid daughters that are semisterile.

The average number of female offspring from the two parental types was equivalent (Table 1, experiment 1). However, the average number of sons differed. There were 8.6 ± 14.84 sons from the uninfected wasps but only 2.1 ± 4.01 sons in the *sk*-infected broods. Thus, the difference in sex ratios is due to a difference in the production of sons rather than daughters. Such a difference could be due to an alteration in the primary (zygotic) sex ratio produced by an ovipositing female or due to an alteration in the secondary sex ratio because of mortality of sons. This is examined in the next experiment.

Experiment 2: cause of the sex ratio skew in sk*-infected broods*: Three mechanisms may be hypothesized to explain the altered sex ratios of *sk*-infected females. (1) The fertilization behavior of infected females is altered, resulting in increased fertilization rates and, hence, a higher proportion of daughters. (2) The morphology or physiology of infected females is altered preventing them from limiting sperm access to eggs. (3) Equivalent primary sex ratios are produced but there is differential mortality of the male embryos in *sk*-infected broods. Since numerous unhatched eggs were observed in *sk*-infected broods, the third hypothesis appeared to be the most likely; the following experiment tests this quantitatively.

For the experiment, infected and cured substrains of the HEB-3 strain were used. The distinction between the substrains was based on three generations of sex ratio data; it has subsequently been confirmed by the lack of reversion to the son-killer phenotype in the cured substrain in more than a year of

TABLE 2

Numbers of hatched and unhatched eggs obtained from virgin females with and without the son-killer factor

Strain	*sk*	Eggs laid (mean)	No. of inviable eggs	No. of viable larvae	% inviable eggs
HEB-3	+	33.3 ± 7.82 (30)	795	205	79.5
HEB-3	−	34.0 ± 8.75 (9)	8	298	2.6
ScDr	−	44.8 ± 6.71 (10)	13	435	2.9

Virgin females lay only male eggs. The counts were made only after all viable eggs would have hatched. Numbers in parentheses are the number of females tested.

intermittent testing. (The term "cured" is appropriate because the HEB-3 strain originated from a single female.) Females of the (uninfected) ScDr strain were used as an additional control.

To assay the mortality of male eggs, virgins of the three strains were allowed to parasitize hosts. [Virgins produce only male progeny and show no inhibition of oviposition (WHITING 1967).] Hosts from each group were opened after all viable eggs would have hatched and the unhatched eggs and first instar larvae were counted.

Table 2 presents the results of the mortality assay. Infected and uninfected females of the HEB-3 substrains laid comparable numbers of eggs, whereas the uninfected ScDr females laid one-third more. [This difference in fecundity is not typical (SKINNER 1983) and probably is due to slight differences in the age of the ovipositing females.] Of the eggs laid, 79.5% failed to hatch in the *sk*-infected broods, whereas, only 2.6 and 2.9% (HEB-3 and ScDr, respectively) failed to hatch in the uninfected broods. Clearly, the two sets of uninfected broods show low and comparable levels of egg mortality, whereas the *sk*-infected broods show significantly more. This figure for male embryo mortality (80%) is comparable to the difference in the production of sons by the mated females in experiment 1 (74%, Table 1). Thus, the exceptional sex ratio distributions of infected females appear to be due solely to the mortality of their male offspring during embryogenesis. No alteration of the primary sex ratio need be postulated to account for these results.

The preceding experimental protocol provides a means of easily distinguishing infected from uninfected females. Note, however, that females are distinguished by the presence or absence of mortality among their male *progeny*. Thus, mortality of offspring is used to categorize females in the preceding *parental* generation.

Experiment 3: maternal transmission of the son-killer factor: A third experiment examined transmission of the son-killer factor between generations. The trait could be due to one or more chromosomal genes or to an extranuclear factor showing a non-Mendelian pattern of inheritance. To distinguish between these possibilities, a series of crosses was made within and between infected and uninfected strains.

TABLE 3

Mean numbers of unhatched male eggs from virgin females in two parental strains and after three generations of backcrossing within and between the strains

	HEB-3 ♀ (*sk*-infected)		ScDr ♀ (uninfected)	
Generation	× HEB-3 ♂	× ScDr ♂	× HEB-3 ♂	× ScDr ♂
Parental	26.1 ± 7.53 (40)			0.3 ± 0.54 (40)
F_4	18.7 ± 13.95*** (80)	21.8 ± 13.86* (80)	0.5 ± 0.76 (80)	0.4 ± 0.71 (80)

Comparisons are between the parental and the F_4 generations and assume that on average equal numbers of eggs were laid.
* $P < 0.05$; *** $P < 0.001$.

Virgin females were isolated from the HEB-3 and ScDr strains and allowed to parasitize hosts. The hosts were examined for the presence of inviable eggs and the females were scored as *sk* infected or uninfected on this basis. The average number of unhatched eggs from the two types of females is given in Table 3 (parental generation). Forty *sk*-infected females from the HEB-3 strain and the 40 uninfected ScDr females were then mated to produce daughters and given fresh hosts. The daughters of each female were sorted into two sublines. One subline was repeatedly backcrossed to males of the same strain, whereas the other was repeatedly backcrossed to males of the alternate strain. After three generations of backcrossing, two virgin females from each subline (F_4 generation) were assayed for the presence of son-killer by the relative number of inviable male eggs.

Repeated backcrossing between strains results in the systematic substitution of the chromosomal genes of one strain by those of the other. If the son-killer trait were chromosomally inherited, sublines that have been backcrossed to HEB-3 males should exhibit high mortality of the male eggs, whereas there should be little mortality in the sublines backcrossed to ScDr males. By contrast, if the trait is maternally inherited, those sublines with a HEB-3 maternal background should continue to show high mortality of male eggs, independent of their nuclear genotype. The sublines with an ScDr maternal background should exhibit little mortality of their male eggs.

The results of the mortality assay in the F_4 generation are also given in Table 3. No female in any subline with an ScDr maternal origin produced the large numbers of inviable eggs that would signal the presence of the son-killer factor—the highest number from a single female was three. Overall, the average number of unhatched eggs was equivalent among the parental cross and the two backcrossed sublines. By contrast, the average number of unhatched eggs was high in both of the sublines with a HEB-3 maternal background. Although the means are equivalent between the two backcrossed lines, both averages are significantly lower than in the parental generation because some females produced few or no inviable eggs (recall that such females were eliminated from the parental set). In ten of the sublines backcrossed to HEB-3

males, neither tested virgin yielded high numbers of inviable eggs. In an additional three sublines, only one of the two virgins assayed showed high egg mortality. The equivalent figures for the sublines backcrossed to ScDr males are seven and four, respectively.

Absence of the son-killer factor in the ScDr ♀ × HEB-3 ♂ backcross is inconsistent with a model based on a chromosomal gene or genes. Furthermore, there is no evidence for paternal transmission of the factor (extrachromosomally), although rare paternal inheritance is not excluded. The entirety of the results is consistent with strict maternal inheritance if the low egg mortality in some clutches with a HEB-3 maternal origin represents incomplete transmission from mother to daughter. Transmission failure appears to be independent of genotype because the frequency of occurrence of females without son-killer is similar regardless of the direction of the backcross.

Experiment 4: rate of transmission of the son-killer factor when maternally inherited: As indicated in experiments 2 and 3, transmission of the son-killer factor is incomplete. An experiment was conducted to estimate the frequency with which it is lost. Parental females were isolated from the HEB-3 strain as virgins and assayed for *sk* infection by the production of high numbers of inviable male eggs. This ensured that only infected females were used to produce daughters. Equal numbers of daughters were then obtained from each parental female and assayed for the production of inviable eggs, indicating inheritance of the son-killer factor.

Of 179 hosts parasitized by the daughters, five had three or fewer unhatched eggs and were scored as uninfected. The remainder had a minimum of nine unhatched eggs, indicating inheritance of son-killer. Thus, by this assay, 97% of the daughters inherited *sk* under these conditions. The (binomial) 95% confidence limits are 91–98% (ROHLF and SOKAL 1969, table W), assuming that the probability of transmission is constant and independent across females. How reliable is this assay? Ten broods scored as uninfected by the preceding assay (in earlier experiments) have shown no reversions to the son-killer phenotype in four or more generations.

Experiment 5: contagious transmission of the son-killer factor: Superparasitism occurs when a female parasitizes a host previously parasitized by another female. The following experiment ascertains whether son-killer can be contagiously transmitted through superparasitism.

For experimental hosts, infected females of the HEB-3 strain were mass mated and then each female was given a host to parasitize (See Figure 2: Primary ♀). After 24 hr, each female was removed and a mass-mated female of the uninfected ScDr strain was then given 24 hr to superparasitize the host (Figure 2: Superparasite ♀). The superparasitizing female's offspring were exposed thereby to offspring of *sk*-infected females. The offspring were distinguishable because of the different eye colors in the two strains. For control hosts, both the primary and the superparasitizing females were from the ScDr strain. Progeny of the superparasitizing female were not exposed to *sk*-infected wasps. The progeny of the two females in these hosts cannot be distinguished, but this was not felt to be important because uninfected females produce

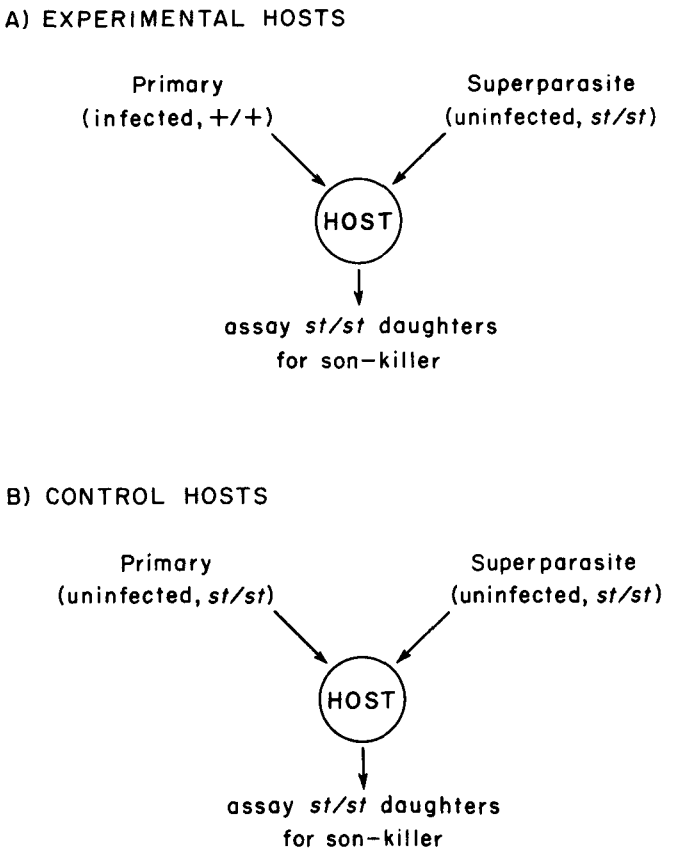

FIGURE 2.—Design of experiment testing for contagious transmission of the son-killer factor in superparasitism. Primary females were given 24 hr to parasitize a host. On being removed, they were replaced with another female that was given 24 hr to superparasitize the host. The generation 1 scarlet-eyed progeny were assayed for son-killer by examining *their* clutches for inviable male eggs and/or obtaining a distribution of sex ratios. In a repeat of this experiment, the eye colors were reversed. See text for details. + = wild-type (brown) eye color allele at the *R* locus; *st* = scarlet eye color allele ($R^{st\text{-}DR}$).

similar sex ratios independently of whether they are from a primary or superparasite brood *per se* (HOLMES 1970). To assay for contagious transmission of son-killer, exposed (ScDr) females from each host were isolated, pair mated and given hosts to obtain sex ratios.

Unexpectedly, there was high mortality among the first generation of *sk*-exposed females with the result that only a small number of sex ratios was obtained. Thus, data from the second generation after exposure are presented in Figure 3a. The sex ratios of the exposed line of ScDr females were significantly lower than those of the unexposed line, despite their equivalent genotypes ($H = 41.25$, d.f. = 1, $N = 103$, $P \ll 0.001$, one-tailed). Data from these broods indicate that the sex ratio effects are due to high mortality of the male offspring (77%, Table 1, experiment 5a) in the exposed line. These data demonstrate contagious transmission of *sk* from the HEB-3 strain to females of the

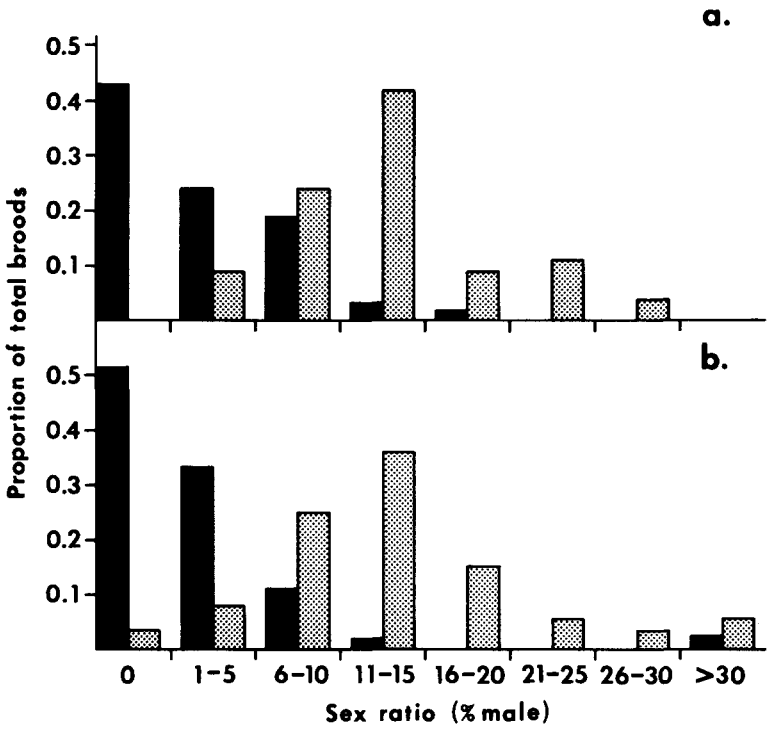

FIGURE 3.—Sex ratio distributions obtained from females exposed to *sk* (solid bars) and from control females not exposed to *sk* (stippled bars). Exposure to son-killer occurred by rearing the females in hosts initially parasitized by an *sk*-infected wasp (see Figure 2). Each female was isolated with a single host for 24 hr. a, Both sets of females are derived from the ScDr strain (*sk*-exposed females, $N = 58$; unexposed females, $N = 45$). b, Females of the cb^+ strain (*sk*-exposed females, $N = 45$; unexposed females, $N = 39$).

ScDr strain. The *sk* factor has been maintained by maternal transmission for more than 15 generations in the newly infected line.

Subsequently, the experiment was repeated using *sk*-infected females from the newly infected ScDr line as primary females in the experimental hosts. Uninfected females from another strain (cb^+) served as primary females on control hosts and as the superparasites. Note that here the eye color genotypes are reversed. The experiment also differed in that contagious transmission was assayed by counting inviable eggs in the clutches of virgins as well as comparing the sex ratio distributions of exposed and unexposed mated females.

In this repetition of the experiment, there was no mortality of the exposed females in the first generation. Sex ratios of *sk*-exposed and unexposed cb^+ females of this generation are plotted in Figure 3b. Exposed females produced significantly lower sex ratios than did unexposed ($H = 44.89$, d.f. $= 1$, $N = 84$, $P \ll 0.001$, one-tailed). Data on offspring numbers from these females also indicate high mortality of sons from the exposed females (89%, Table 1, experiment 5b). These results are consistent with acquisition of the son-killer factor by contagious transmission. Additionally, 74 *sk*-exposed cb^+ virgins were

assayed for *sk* by counting their inviable eggs. Of these, 72 were scored as infected, yielding a transmission rate of 97%. The unexposed virgins that were assayed ($n = 20$) laid very few inviable eggs, consistent with the absence of infection.

Limited additional experiments (using the original infected HEB-3 and uninfected ScDr strains) indicate that contagious transmission occurs even when the *sk*-infected female serves as the superparasite. It is independent of whether she is a virgin or has been mated. The unexpected female mortality observed in the first generation of the newly infected ScDr line (experiment 5a) has not occurred in these additional experiments. Therefore, if the mortality in experiment 5a proves repeatable, it is specific to the strains used and the sequence of exposure.

DISCUSSION

The son-killer trait is maternally and contagiously transmitted, altering the sex ratio of infected females through mortality of their male offspring. Approximately 95% of the daughters of an infected female inherit the trait. Similarly, in contagious transmission (between infected and uninfected clutches) on superparasitized hosts, approximately 95% of the daughters from the uninfected clutch acquire the trait. What mechanistic questions are raised by these results and what are the evolutionary implications of the son-killer and related traits?

One set of mechanistic questions concerns the mortality of males. How are they killed and at what stage of embryogenesis? How is the mortality limited to males? In some Drosophila species, sex ratio organisms (a spiroplasma and an associated virus) kill males early in embryogenesis. It is hypothesized that the mortality is due to male susceptibility to an androcidin that the virus is postulated to produce (WILLIAMSON and POULSON 1979). However, since individuals with two or more *X* chromosomes survive regardless of the phenotypic sex, maleness *per se* is apparently not involved. KOANA and MIYAKE (1983) suggest that susceptibility depends strictly upon the number of *X* chromosomes in the genome.

The actual cause of mortality of Nasonia males is unknown. However, if, even during vertical transmission, the son-killer factor is transmitted through the host's hemolymph rather than the eggs as is suggested below, then an androcidin is a likely candidate. It would be necessary for the androcidin to be present in the eggs prior to laying rather than being produced by the factor during male embryogenesis. There are three possibilities for how mortality is limited to males. The distinction may be made between fertilized and unfertilized eggs, or by ploidy, or by maleness *per se*. These alternatives are testable.

Contagious transmission of the son-killer factor indicates that the factor is not only extrachromosomal but is also extracellular, at least during some stage in the Nasonia life cycle. The similarity of the transmission rate from mother to daughter and in contagion is striking; in both cases, approximately 95% of the females inherited or acquired the son-killer factor. This congruence suggests that a similar mechanism may be involved in both. If we consider the

possible mechanisms for contagious transmission of *sk* in superparasitism, there are two possibilities: via contact between infected and uninfected offspring (as eggs, larvae or pupae) or via feeding on infected hemolymph of the host. Of these, the second actually *predicts* that the rates of mother-to-daughter and contagious transmission should be similar since the parental source of the feeding larvae *per se* is unimportant.

Thus, it is possible that *sk* is transmitted by being injected into the host hemolymph during parasitization by an infected female (perhaps when the female injects her venom to kill the host) and is then acquired by her offspring (and any others, as well) during larval feeding. Why some individuals escape infection is unclear. However, it is unlikely to be due to genetic differences among wasps because the strains used are highly isogenic.

Naturally occurring contagious transmission was not observed for the extrachromosomal sex ratio factors found in *Drosophila prosaltans* (CAVALCANTI and FALCAO 1954) or in the mosquito, *Culex tarsalis* (KELLEN and WILLS 1962).

Extrachromosomal factors affecting sex allocation are known in several taxonomic groups. In plants, cytoplasmic male sterility is reported in more than 100 species (EDWARDSON 1970). The trait is due to pollen abortion during development (LASER and LERSTEN 1972). Sex-converting factors are suspected in a number of Crustacea (GINSBURGER-VOGEL 1973) and have been well studied in several species (GINSBURGER-VOGEL 1975; JOHNSON 1977; BULNHEIM 1978; JUCHAULT and LEGRAND 1981; BULL 1983). Sex differentiation in normal males of these species is mediated by hormones released from the androgenic gland. In infected males, the cytoplasmic factors interfere with or suppress the functioning of this gland, causing genotypic males to develop as fully functional females that transmit the factor to their offspring.

With the exception of the *psr* and *msr* factors in Nasonia, all extrachromosomally induced sex ratio phenomena in insects are of the sex-killing type, causing mortality of the male offspring. This contrasts with crustaceans. Concurrent with this is a difference in the sex differentiation mechanisms of the two taxa. In insects, sex differentiation is not hormonally mediated but occurs on a cell autonomous basis with each cell differentiating according to its own genotype (see, *e.g.*, BAKER and RIDGE 1980). It is not clear whether these differences between the taxa are associated. However, it is interesting that species of the microsporidian genus Thelohania kill males in certain mosquitos, but *T. herediteria* converts males into females in a crustacean (BULNHEIM 1975).

From an evolutionary perspective, extrachromosomal factors affecting sex allocation are best viewed as "parasites" or even "diseases" of their "hosts." This view emphasizes the differences in selective pressures acting on the two, differences that come about because of the differences in their modes of inheritance. A major question is how such factors are maintained in natural populations, or, phrased differently, what are the selective advantages to altering sex allocation patterns?

Sex-converting factors such as those in crustaceans or both the paternal sex ratio and maternal sex ratio factors in Nasonia alter the primary sex ratio produced by a female. In doing so, they intrinsically acquire a fitness advantage analogous to that observed in meiotic drive (SANDLER and NOVITSKI 1957), in

selfing (WELLS 1979) or in parthenogenesis (WILLIAMS 1975; MAYNARD SMITH 1978). This advantage arises because of the favorable bias in their transmission from one generation to the next. By contrast, the son-killer factor and all other factors observed in insects only affect the secondary sex ratio in a clutch. They do not affect sex allocation *sensu stricto*; but nonetheless, it is convenient to consider them in this context. Killing males *per se* is of no selective advantage to a maternally inherited factor. Hence, in developing an hypothesis of the selective advantage for such a trait some concomitant effect on fitness must be sought (SKINNER 1983). Works by LEWIS (1941) and IKEDA (1970) are rare examples of attention to this problem.

For the *sk* factor in Nasonia, the problem is pertinent because the trait occurs at a low but significant frequency in natural populations of the wasp (~4%, SKINNER 1983). Also, it can increase in frequency when introduced into new stocks as a rare "mutant" (S. W. SKINNER, data not presented).

Two nonmutually exclusive advantages to the killing of males may be suggested. (1) An inverse relationship exists between the number of offspring developing on a host (of a given species and size) and the size of those offspring at emergence (CHARNOV and SKINNER 1984). Furthermore, there is a positive relationship between a female's size and fecundity (KING and HOPKINS 1963; CHARNOV and SKINNER 1984). In light of these empirical relationships, it may be suggested that son-killer is selected to kill male embryos because this frees additional food for the daughters (that transmit the factor) enabling them to grow to a larger, more "fit" size. Note that, for the parental wasp, this mortality is of no advantage because the gain through daughters comes at the expense of representation through sons.

(2) A second hypothesis is that the mortality of sons is associated with contagious transmission of *sk*. If mortality were a necessary antecedent for contagion or even if it only increased the probability of such transmission given that superparasitism has occurred, the killing of males would be favored because of the increased transmission rate. However, the suggestion that *sk* is passed through the host hemolymph runs counter to this hypothesis for Nasonia.

The occurrence of three separate factors in a single species, each with a different mechanism for altering the sex ratio, is unique. Moreover, all three factors occur at significant frequencies in wild populations (SKINNER 1983). Thus, they represent natural phenomena of evolutionary significance to the wasp. Combined with the behavioral variations in the sex ratios of females, this yields a singularly complex set of phenomena. Despite this complexity, the system is quite tractable to experimental study and should provide useful insights into sex allocation problems from both mechanistic and evolutionary perspectives.

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