Sex Ratio Adaptations to Local Mate Competition in a Parasitic Wasp

Abstract. *Females of the parasitic wasp* Nasonia vitripennis *adjust the sex ratio of their broods according to whether they are a first or second wasp to parasitize a host. The first wasp produces a strong daughter bias. The second wasp adjusts the proportion of sons to the relative level of local mate competition, as predicted by a natural selection model. The results provide a quantitative test of sex ratio theory.*

Sex ratio evolution has been considered theoretically and empirically ever since Darwin posed the problem (*1*). Two fundamental developments are Fisher's theory (*2*), which predicts "equal investment" in the sexes for random mating populations (usually implying a 50 : 50 primary sex ratio), and Hamilton's local mate competition (LMC) theory (*3*), which predicts a female-biased sex ratio in species where sons of a parent compete with each other for mates. Usually LMC occurs in small mating populations with low male dispersal and is exemplified by various arthropod species for which sibmating is common. Selection favors a parent to bias sex ratio toward daughters because fitness returns from sons are less than linearly increasing with the number of sons produced. Data from some sibmating species are generally in qualitative agreement with the theory (*3, 4*). In this report I extend Hamilton's LMC theory by adding the realistic assumption that broods contributing to local mating populations may differ in size. This simple alteration leads to different quantitative predictions, which are tested with the parasitic wasp *Nasonia vitripennis*. The results fit well and provide one of the few quantitative tests of any sex ratio theory. The model also has applications well beyond the present experimental system.

Nasonia vitripennis is a small (~ 3 mm) chalcidoid wasp that parasitizes cyclorrhaphous fly pupae (*5, 6*). Like most other Hymenoptera, *N. vitripennis* has haplodiploid sex determination; unfertilized (haploid) eggs develop into males, and fertilized (diploid) eggs develop into females. Internal structures in *Nasonia* appear to regulate fertilization (*7*) and, therefore, the sex ratio. When a female wasp encounters a host pupa, she drills through the puparial wall, kills the host by stinging, and lays eggs on it (20 to 40 on *Sarcophaga bullata*). The average sex ratio per host is strongly female biased; 8.7 percent sons (S.D., 4.6 percent, $N = 95$) among an average offspring number of 28.6 (S.D., 11.3, $N = 95$) (*8*). Development to adulthood occurs in 14 days at 25°C. No mating occurs within the puparium (*5, 6*), but males emerge from the puparium first and contest for proximity to the exit hole. Emerging females mate immediately with the waiting males and then disperse. Males are capable of inseminating numerous females and females normally copulate only once (*9*). The males are relatively short-lived (*10*) and cannot fly because they have vestigial wings. Therefore, strict sib-sib mating will occur unless (i) males arrive from nearby hosts parasitized by a different wasp or (ii) a second wasp lays eggs upon the same host (termed superparasitism).

The second wasp to attack a host can detect previous parasitization. It has been shown (*11*) that females laying eggs on previously parasitized hosts (i) lay fewer eggs and (ii) lay a greater proportion of sons than do females on unparasitized hosts. The greater proportion of sons cannot be explained by increased mortality of female offspring (*11*), nor can it be explained as the wasp laying the same number of male eggs, but withholding female eggs (*12*). Hamilton (*3*) proposed that this "superparasitism" response was a facultative sex ratio adaptation since the decreased LMC in superparasitized hosts would favor an increased production of male offspring. His quantitative model assumed that both females produced broods of equal size.

However, brood size may vary. The following model assumes (i) females mate only with males from their own host; that is, there is no male movement between hosts; (ii) the first and second broods emerge synchronously and mate randomly; (iii) the second brood does not suffer increased mortality; (iv) there is no inbreeding depression; and (v) the second wasp can vary her sex ratio. These assumptions are discussed below.

Let T equal the number of eggs laid by the second female per number of eggs laid by the first female; X_1 is the proportion of sons laid by the first female; X_2 is

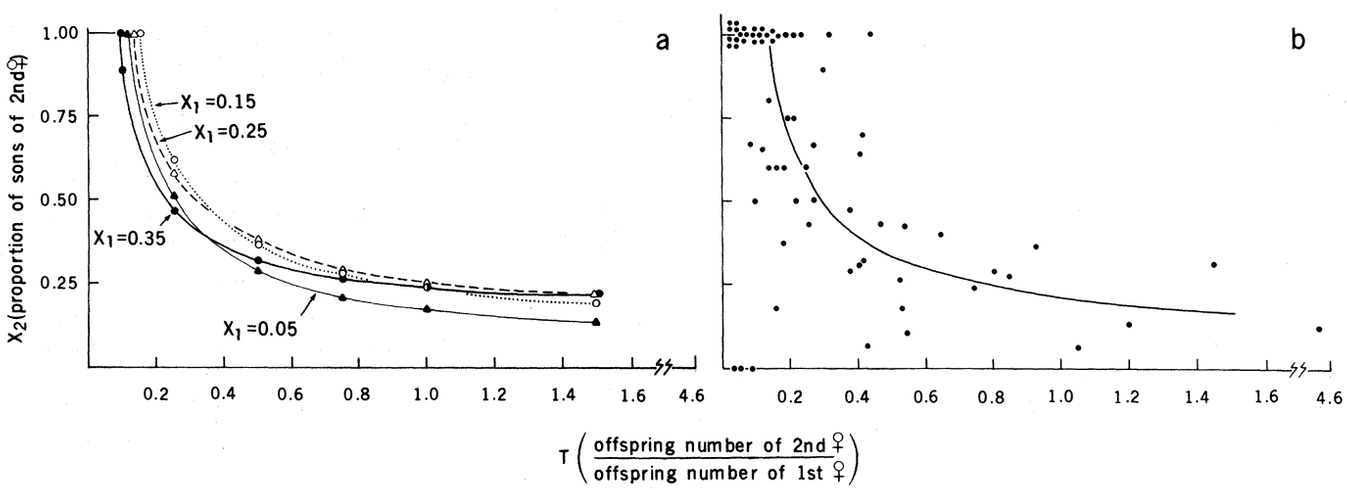

Fig. 1. Sex ratio dynamics of the parasitic wasp *Nasonia vitripennis* in response to varying level of local mate competition. (a) The optimal proportion of sons of the second female to parasitize a host as a function of her relative egg number for different values of X_1 (first female's sex ratio) and T (relative egg number). (b) The observed proportion of sons of the second female as a function of relative number of emerging offspring. The expected curve based on the average sex ratio of the first female ($X_1 = 0.087$) is plotted in Fig. 1b for reference. The points clustered about $X_2 = 1.0$ all fall on $X_2 = 1.0$.

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the proportion of sons laid by the second female; W is the fitness of the second female; X_2^* is the proportion of sons laid by the second female which maximizes her fitness. In the manner of Hamilton (*3*), the fitness of the second female can be represented by

$$W = \underbrace{\frac{TX_2}{X_1 + TX_2}\,[1 - X_1 + T(1 - X_2)]}_{\text{(from sons)}} + \underbrace{T(1 - X_2)}_{\text{(from daughters)}} \quad (1)$$

A maximum of W occurs when

$$X_2^* = \frac{(\sqrt{2X_1(T + 1)} - 2X_1)}{2T} \quad (2)$$

Since X_2^* depends on X_1 (as well as on T), it is relevant to consider the optimum sex ratio of the first female. Her optimum will depend on the probability of superparasitism and the frequency distribution of superparasitism level (T). This is more complicated, but the minimum and maximum X_1 can easily be found. When superparasitism never occurs, the first female should produce only enough sons to inseminate daughters. When superparasitism is certain and T always equals 1.0, the first female optimum is only 0.25 sons (*13*). Thus, the first female optimum should fall between 0 and 0.25 proportion of sons.

Figure 1a presents the optimum second female sex ratio as a function of T for four different first female sex ratios, ranging in value from 0.05 to 0.35 sons. These curves show that the second female optimum is rather insensitive to the first female sex ratio. The relative egg number (T) is the important variable determining the second female optimum. When the second female lays relatively few eggs she should produce 100 percent sons. As the relative egg number of the second wasp increases, the optimum proportion of sons first drops rapidly and then declines less rapidly, such that 19 to 25 percent sons is optimal when both the first and second wasps lay the same number of eggs. This arises because when the second wasp lays few eggs, her sons do not compete with each other but have high mating success owing to the large number of daughters produced by the first wasp. As her egg number increases, sons face increasing local mate competition, favoring a more female-biased sex ratio (*14*).

The assumptions of the model appear to be reasonable. (i) Field observations indicate that isolated hosts are common in nature because fly larvae often crawl long distances (up to 15 m) from a carcass before pupating. (ii) Experimental observations show that males from both the first and second broods emerge synchronously, even when the second parasitization occurs 48 hours after the first. Synchrony in male emergence is enhanced because males develop more rapidly than females and because the eclosed wasps remain within the host for more than 24 hours before emergence and mating. Using genetic markers, I have observed that mating readily occurs between the two broods, although it has not been determined whether it is random (*15*). (iii) Comparisons of egg counts to emergences show that mortality is low in superparasitized blowfly pupae (*16*). (iv) *Nasonia* also readily inbreeds without deleterious effects (*6*). (v) In *Nasonia* anatomical structures exist that could provide control over the sex ratio (*7*), and experiments amply show that some control is exercised (*11*).

To test the model, a single wild (+) or scarlet eye (ScDr) genotype female (*17*) was placed into an arena with four *Sarcophaga bullata* hosts. Both genotypes are highly viable, and no relevant sex ratio differences were observed. After 24 hours the arena female was removed, and the alternative genotype was placed into the arena and left for 24 hours to superparasitize the hosts. The number, sex, and genotype of emerging offspring were recorded (*18*) (Fig. 1b). The expected curve based on the average sex ratio of the first female ($X_1 = 0.087$) is plotted for reference.

The sex-ratio data show the trend predicted by Eq. 2. When the second female produced relatively few offspring, usually 100 percent were sons; and, as the relative number of offspring increased, the proportion of sons decreased in the manner predicted. A Spearman rank correlation shows a negative correlation between X_2 and T significant at the .001 level ($N = 68$).

This very small wasp, *N. vitripennis*, is thus apparently capable of pursuing a fairly subtle sex ratio strategy. The female alters the sex of her offspring in a manner that increases her fitness under varying pressures of local mate competition. The parameters influencing sex ratio changes (for example, whether the second wasp uses host size information) are being investigated.

Finally, the model has implications for mating systems beyond the wasp system for which it was developed. A general prediction is: For organisms that mate in small local populations (but disperse beyond them), those individuals making a relatively small reproductive contribution to the mating population should bias investment more toward male function (sons, pollen, or sperm) and those with a relatively large reproductive contribution should bias investment toward female function (daughters, seeds, or eggs). This concept can be applied to both dioecious and hermaphroditic species (*19*). For example, social insects that mate in neighborhood swarms (*20*) and plants with local pollen flow (*21*) should show the relative LMC effect.

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References and Notes

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8. These data were obtained by placing single female wasps in an arena with five *Sarcophaga bullata* hosts for 24 or 48 hours. The arena is an enclosed tissue culture tray with 24 wells. Wasp reproduction was not limited by host availability in the time allotted.
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12. To test this, wasps were first provided with hosts for 24 hours and then individuals were isolated in test tubes and provided with honey for another 24 hours. Each was then presented an experimental host for 3 hours. For the superparasitism group, the host had been parasitized by a similarly treated ScDr female for 3 hours, 24 hours before presentation to the wild-type female. A control group received an unparasitized host. From the superparasitized hosts ($N = 29$), 5.03 ± 3.8 wild-type males and 9.03 ± 6.1 wild-type females emerged versus 2.09 ± 2.35 males and 16.6 ± 7.5 females in the control hosts ($N = 34$). The wild-type female laid significantly more sons in the superparasitized host (t-test, $P < .005$).
13. This is the same optimum as derived from Hamilton's model, which assumed $T = 1.0$ (*3*).
14. H. B. Holmes [thesis, University of Massachusetts (1970)] noted that superparasitizing wasps that lay few eggs should produce a high proportion of sons, but gave no quantitative theory. My model neglects the inbreeding effect in haplodiploid organisms (as distinct from LMC or inbreeding depression) on sex ratio selection. This effect is probably minor (*4*).
15. Frequency-dependent mate selection has been found in some strains of *N. vitripennis* [B. Grant, G. A. Snyder, S. F. Glessner, *Evolution* **28**, 256 (1974); H. C. White and B. Grant, *ibid.* **31**, 829 (1977)]. It is not known how general the effect is nor whether it is expressed when different genotypes are reared in the same host.
16. Wasps were first treated as in (*12*), and then placed singly in an arena with four dispersed hosts for 12 hours. An ScDr female was placed first in the arena. Thirty-six hours after her removal, a wild-type female was placed into the arena. For egg counts, the hosts were opened 12 hours after removal of the second female. This allowed determination of the second brood eggs because eggs from the first brood had already hatched. The mean egg number (3.80 ± 5.11; $N = 80$) does not differ significantly (t-test, $P > .40$) from emergence number (3.58 ± 5.51, $N = 79$). Neither are the distributions significantly different ($\chi^2 = 2.8$; d.f. = 4).
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18. The wasps were treated as in (*12*) before being

placed in the arenas. Occasionally the first or second female was upon a host when she was removed from the arena. These hosts are not included in Fig. 1b since the female may not have completed oviposition.

19. Other theories predict that circumstances can favor small (or young) individuals to bias investment to male function. This is favored, for example, when reproductive success increases with size in females, but does not increase with size in males [R. R. Warner, *Am. Nat.* **99**, 419 (1975); E. L. Charnov, *ibid.* **113**, 715 (1979)]. The relative LMC model presented in this paper shows that a size effect results from LMC itself. E. L. Charnov has found that similar arguments apply to barnacles (personal communication).
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22. I thank E. L. Charnov for financial support; J. J. Bull, M. R. Gross, S. W. Skinner, and E. L. Charnov for helpful discussions; J. van den Assem for teaching me about wasps; and W. D. Hamilton for his generous assistance. Supported in part by NSF grant DEB 7683011 A01 and NIH grant 5T32 GM0746402.

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Reactivation of Infant Memory

Abstract. *Three-month-old infants learned to activate a crib mobile by means of operant footkicks. Retention of the conditioned response was assessed during a cued recall test with the nonmoving mobile. Although forgetting is typically complete after an 8-day retention interval, infants who received a reactivation treatment—a brief exposure to the reinforcer 24 hours before retention testing—showed no forgetting after retention intervals of either 2 or 4 weeks. Further, the forgetting function after a reactivation treatment did not differ from the original forgetting function. These experiments demonstrate that (i) "reactivation" or "reinstatement" is an effective mechanism by which early experiences can continue to influence behavior over lengthy intervals and (ii) memory deficits in young infants are best viewed as retrieval deficits.*

The pervasive influences of early experiences on later behavior have been extensively documented, as have early memory deficits or "infantile amnesia" (*1*). Considered jointly, these phenomena pose a major paradox for students of development: How can the effects of early experiences persist into adolescence and adulthood if they are forgotten during infancy and early childhood? Campbell and Jaynes (*2*) proposed a resolution to this paradox in terms of reinstatement, a mechanism that maintains a memory which would otherwise be forgotten through occasional reencounters with the original training conditions over the period of development. Any given reencounter, however, would be insufficient to promote new learning in organisms lacking the early experience. Spear (*3*) attributed the efficacy of reinstatement procedures to improved retrieval produced by the reactivation of a sufficient number (or kind) of existing but otherwise inaccessible attributes of the target memory. He hypothesized that reexposure to stimuli from the original training context, which had been stored as attributes of the memory, could prime or arouse other attributes that represented the original experience, increasing their accessibility and, thus, the probability of their retrieval.

"Reinstatement" or "reactivation" has been demonstrated in young and adult rats (*2*, *4*, *5*) and in grade-school children (*6*). We now report that a reactivation treatment can alleviate forgetting in 3-month-old infants after a retention interval as long as 4 weeks and that the forgetting function after a reactivation treatment is similar to the function after original training.

Our procedures were modeled after those of animal memory studies in which the experimenter trains a specific response in a distinctive context and later returns the subject to that context to see if the response is still produced. Because the retrieval cues are contextual and response production is assessed before reinforcement is reintroduced, the procedure is analogous to a test of cued recall (*3*).

In our studies, footkicks of 3-month-olds were reinforced by movement of an overhead crib mobile. The infant controlled both the intensity and frequency of the mobile movement by means of a ribbon connecting the ankle (Fig. 1A) with the hook from which the mobile hung. This procedure, "mobile conjugate reinforcement," produces rapid acquisition and high, stable response rates attributable to the contingency and not to behavioral arousal (*7*). During nonreinforcement phases (baseline, retention tests, extinction), the mobile remained in view but was hung from a second mobile stand with no ribbon attachment and could not be activated by kicks.

Infants received three procedurally identical sessions in their home cribs. The first two were training sessions, spaced by 24 hours; the third followed a lengthy retention interval. Each session consisted of a 9-minute reinforcement phase preceded and followed by a 3-minute nonreinforcement period. In session

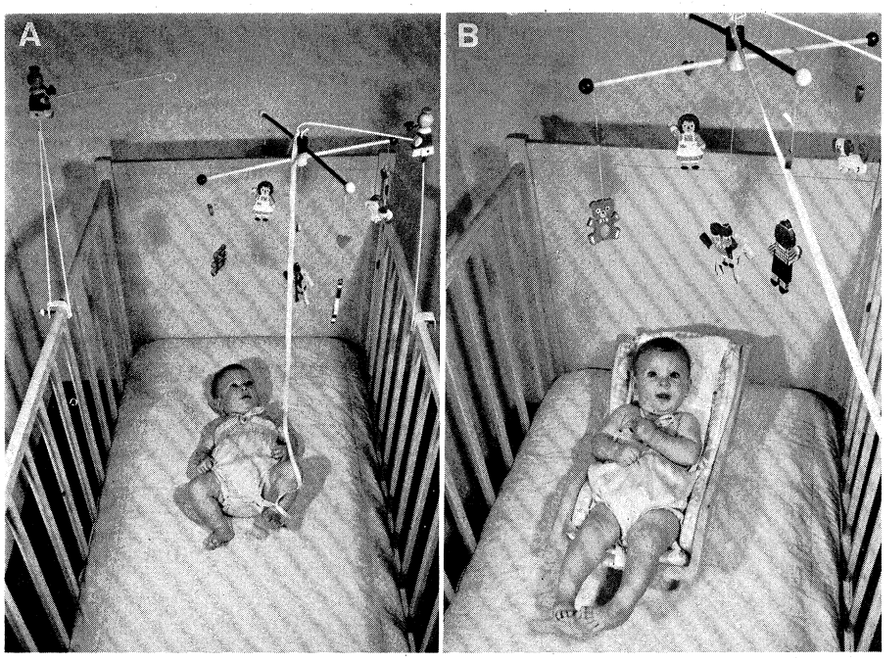

Fig. 1. (A) An infant during a reinforcement phase with the ankle ribbon attached to the same suspension bar as that from which the mobile hangs. The empty mobile stand, clamped to the crib rail at the left, will hold the mobile during periods of nonreinforcement. (B) The same infant during a reactivation treatment. The mobile and ribbon are attached to the same suspension hook, but the ribbon is drawn and released by the experimenter (not shown), concealed from the infant's view at the side of the crib. Also not shown is the empty stand, positioned as before. The infant will be exposed to the reinforcer (the moving mobile) for only 3 minutes 24 hours before retention testing. [Photograph by Breck P. Kent]

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