

King, B.H. and S.W. Skinner. 1991. Sex ratio in a new species of *Nasonia* with fully-winged males. *Evolution* 45:225-228.

This version matches the text but not the formatting of the published article.

SEX RATIO IN A NEW SPECIES OF *NASONIA* WITH FULLY-WINGED MALES

B. H. KING¹ AND S. W. SKINNER

Department of Biology, Indiana University, Bloomington, IN 47405.

¹Present address: Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115.

One of the better-studied sex ratio theories is local mate competition (LMC) theory, first developed by Hamilton in 1967 (for reviews see Charnov, 1982; Waage, 1986; King, 1987). LMC models assume a subdivided population structure with emergence and then random mating taking place within local patches followed by female dispersal to new patches to lay offspring. Under these conditions, offspring sex ratio (proportion sons) is expected to increase with increasing number of ovipositing mothers in a patch, eventually reaching an asymptote (Hamilton, 1967, 1979).

One of the best-studied species with regards to LMC theory is the parasitoid wasp *Nasonia vitripennis*. *N. vitripennis*'s sex ratio as it relates to LMC theory has been examined both genetically and behaviorally, both in the laboratory and in the field (e.g., Orzack and Parker, 1986; Werren, 1980, 1983). Although current sex ratio theory cannot completely explain offspring sex ratios in *N. vitripennis* (e.g., Orzack, 1986), empirical evidence on *N. vitripennis* supports LMC model predictions in a general way. For example, most *N. vitripennis* females produce female-biased offspring sex ratios when alone and increase the proportion of sons they produce when other ovipositing females are present (Walker, 1967; Wylie, 1965; Velthuis et al., 1965; Werren, 1983).

Here we examine offspring sex ratios in a newly discovered species of *Nasonia*, *N. giraulti* (Darling and Werren, in press), and compare it to that of *N. vitripennis*. In *N. vitripennis*, the explanation for why the species meets the population structure assumed by most LMC models has been that 1) males lack full wings; 2) thus males cannot disperse to mate, and so mating must take place at the emergence site; 3) because mating takes place at the site of emergence, there will be competition for mates among males emerging from the same or nearby hosts (i.e., "local mate competition"). *N. giraulti* is morphologically almost identical to *N. vitripennis*, except that *N. giraulti* males have full wings and can fly. Thus, our expectation was that in *N. giraulti*, males disperse and so their populations, relative to *N. vitripennis*, should experience both less competition among brothers for mates and less inbreeding. Either or both of which should have selected for less female-biased sex

ratios in *N. giraulti* than in *N. vitripennis* (Frank, 1985; Herre, 1985).

We present results showing that *N. giraulti* does manipulate offspring sex ratio in response to number of other mothers present, but that *N. giraulti* produces even more, not less, female-biased sex ratios than *N. vitripennis*.

METHODS

A laboratory colony of *N. giraulti* was established from wasps in parasitized *Protocalliphora* sp. found in a bluebird nest in Monroe county, Indiana, U.S.A. in 1986. The *N. vitripennis* used in this experiment were of the scarlet-eye strain (Saul et al., 1965). Colony maintenance and experiments were at 24 L using *Sarcophaga bullata* as hosts (Skinner, 1985).

Laboratory experiments were used to compare the sex ratios of the two species and their responses to the number of females ovipositing in a patch. Offspring sex ratios of groups of one, two, four, eight, and sixteen mated females were determined for both wasp species under the same conditions. Females were grouped in a shell vial and given four hosts for 24 hours. Prior to use, females had been given honey for 24 hours and then honey and a host for a second 24 hours.

To assess the effect of oviposition by multiple females on offspring size, and hence indirectly to assess mortality due to crowding (Werren, 1983), we measured head widths, forewing lengths, and body lengths for a subset of *N. giraulti* offspring, using a digitizing tablet and the Sigma-Scan program. In analyzing the effect of treatment on offspring size, we used vial as the sampling unit to avoid problems associated with lack of statistical independence among offspring within a vial.

RESULTS

Forty-two *Protocalliphora* pupae were collected from the bluebird's nest. *N. giraulti* emerged from twenty-one of these and flies from fifteen. Data were collected from nineteen of the twenty-one parasitized pupae. The clutch size ranged from 4 to 24 wasps per host, averaging 13.6 ± 5.2 SD. Less than one percent of the wasps were in larval diapause and three percent were not sexable. The proportion males in a host ranged from 0.0 to 0.17, averaging 0.07 ± 0.05 SD.

In the laboratory, for both *N. giraulti* and *N. vitripennis*, the proportion of sons increased with increasing number of females (Table 1, $r_s = 1.00$, $P = 0.01$). For *N. giraulti* the increase resulted from both an increase in sons and a decrease in daughters per mother (Table 1). The same pattern was evident in *N. vitripennis* except that the number of sons decreased in the "16-female" treatment.

For every treatment *N. giraulti* produced significantly more female-biased sex ratios than *N. vitripennis* with no overlap between the two species. The sex ratio difference between the species is largely due to lower numbers of sons in *N. giraulti* (Table 1).

There were no significant differences in the proportion of diapaused offspring among treatments for either *N. giraulti* or *N. vitripennis* (Kruskal-Wallis one-way ANOVA, $H = 5.44$, $P = 0.25$; $H = 1.96$, $P = 0.74$). Across treatments, the proportion of diapaused offspring averaged 0.38 ± 0.39 SD for *N. giraulti* and 0.00 ± 0.01 SD for *N. vitripennis*.

With increasing number of ovipositing *N. giraulti* females per vial, number of offspring per host increased ($R^2 = 0.79480$, $P < 0.0000$), and offspring head width decreased (males: $R^2 = 0.25$, $P < 0.001$; females: $R^2 = 0.35$, $P < 0.001$). Head width was positively related to forewing length and body length for both males ($r_s = 0.80$, $P < 0.001$; $r_s = 0.77$, $P < 0.001$) and females ($r_s = 0.81$, $P < 0.001$; $r_s = 0.80$, $P < 0.001$).

DISCUSSION

Relationship Between Number of Mothers and Sex Ratio

For *N. vitripennis* the pattern of a greater proportion of sons when more females are present is consistent with findings of four earlier studies and with the major qualitative prediction of LMC theory (Walker, 1967; Wylie, 1965; Velthuis et al., 1965; Werren, 1983). Our study used the same strain of *N. vitripennis*, the same host species, and the same number of hosts per treatment as Werren (1983). Yet Werren found a smaller increase in proportion sons. This appears to result from his oviposition chamber design, an inverted funnel with a "collecting" vial on top. Females were aspirated out of the vial at four to six hour intervals. The rationale was to prevent overparasitization "by allowing wasps free movements from a patch of hosts after eggs had been deposited" (Werren, 1983). However, females move readily in and out of the vial between aspirations, even before visiting the hosts and even when wasp densities are low (pers. obs.). Thus, some females may be removed without contributing offspring; and, over the course of the experiment, the total number of females in the patch is decreasing. This creates a problem because LMC theory predicts sex ratio based on the number of females ovipositing in a patch. Werren (1983) used the original number of females in a treatment to calculate predicted sex ratio; however, for the reasons just given, this may be an overestimate. Whether females are presented hosts in a set-up similar to Werren's (1983) or are all confined together throughout the experiment appears to affect the results quantitatively but not qualitatively. With both designs a greater proportion of sons were produced when more mothers were present.

In *N. vitripennis* the greater proportion of sons in response to more mothers appears to be a result of maternal manipulation of offspring sex ratio, rather than a result of differential mortality of the sexes (Wylie, 1966; Walker, 1967). Likewise, in *N. giraulti*, given the increase in number of sons per mother in our data (Table 1), it seems unlikely that greater mortality of daughters than of sons due to crowding is sufficient to explain the sex ratio response of *N. giraulti* to other females.

LMC theory is generally used to explain a greater proportion of sons with increasing numbers of ovipositing females. This same pattern, however, can also be produced by another sex ratio model, the variation in fitness (VIF) model (sensu Taylor, in press). The VIF model is an extension of Charnov et al.'s (1981) host quality model. The general idea is that 1) more ovipositing females will result in more offspring per host, 2) which will result in smaller offspring, and 3) being small will be more detrimental to the reproductive success of females than of males. 1) and 2) are true for both *Nasonia* species (this study; Werren, 1983). However, the relative

effect of size (or of crowding directly) on female versus male reproductive success is not known. Thus the applicability of the VIF model to *N. giraulti* and *N. vitripennis* remains to be determined. *N. giraulti*'s sex ratio pattern is not explainable by a pure VIF model, i.e. with no LMC. The pure VIF model predicts an overall male-biased sex ratio (Charnov et al., 1981), yet *N. giraulti*'s sex ratio is consistently female-biased in both the laboratory and the field.

More Female-biased Sex Ratio of *N. giraulti* than of *N. vitripennis*

The more female-biased sex ratios of *N. giraulti* than of *N. vitripennis* was contrary to our expectations (see introduction) and its cause remains to be determined. We suggest five possible hypotheses which warrant testing.

1) Since our data reflect secondary rather than primary sex ratios, one possibility is that the pattern of egg-to-adult mortality differs between the two species. In the absence of further data, this cannot be rigorously excluded. However, sizes of *N. giraulti* progeny from "16-female" patches are well within the range of viability, and Werren (1983) found little evidence for mortality in *N. vitripennis* with comparable offspring numbers.

2) The very female-biased sex ratio of *N. giraulti* may result from a maternally-transmitted sex-ratio factor, such as have been found in *N. vitripennis* (Skinner, 1982, 1985). One factor is a bacterium (son-killer) that kills the male embryos of infected females, resulting in the production of very daughter-biased broods (Skinner, 1985). Based on the high viability of male embryos and on the lack of signal when females are probed with a son-killer specific RNA probe (Skinner et al., MS), we conclude that this strain of *N. giraulti* is free of infection. Another factor, *msr*, causes the production of about three percent sons without causing male mortality (Skinner, 1982, 1983). Experiments are underway to determine if *msr* is present in this strain of *N. giraulti*. The *N. vitripennis* strain used in this study is known to be free of these factors (Skinner 1982, 1985).

3) Our assumption that male *N. giraulti* are more dispersive than *N. vitripennis* may be incorrect. We know that both male and female *N. giraulti* do fly. However, actual dispersal patterns in nature are not known for *N. giraulti*.

4) Another possibility is that in nature *N. giraulti* females are less synchronous in their parasitization of hosts than *N. vitripennis*. Greater asynchrony in parasitization selects for production of more female-biased sex ratios (Nunney and Luck, 1988).

5) Lastly, the VIF model may be more important for *N. vitripennis* than for *N. giraulti*. LMC theory predicts very female-biased sex ratios. However, if both VIF and LMC models are applicable, VIF can reduce the degree of female bias, assuming that size has a more positive effect on female than on male fitness (Charnov et al., 1981; Taylor, in press). This is a common assumption, though one for which there are few data (Charnov et al., 1981; Jones, 1982; King, 1988; van den Assem et al., 1989).

Our results indicate important similarities and differences in the sex ratios of these two species. In both species, females respond to the presence of other females by increasing their proportion sons. However, under equivalent

circumstances, *N. giraulti* females produce significantly more daughter-biased sex ratios than do *N. vitripennis* females. Both the proximate and ultimate causes of these patterns are unknown, but further comparisons of their behavioral, genetic, and ecological underpinnings should provide important insights into sex ratio evolution in these species and parasitic hymenoptera in general.

ACKNOWLEDGMENTS

We thank M. Zellar and J. Dutton for their help with counting and colony maintenance, R. Madej for help with measuring, C. Darling for identifying *Nasonia giraulti*. BHK thanks R. King for helpful discussions on this work. This work was supported by an Indiana University Postdoctoral Fellowship to BHK and by NSF grant BNS 8820069 to SWS and BHK.

LITERATURE CITED

- ASSEM, J. VAN DEN, J. J. A. VAN IERSEL, AND R. L. LOS-DEN HARTOGH. 1989. Is being large more important for female than for male parasitic wasps? *Behavior* 108:160-195.
- CHARNOV, E.L. 1982. *The Theory of Sex Allocation*. Princeton Univ. Press, Princeton, NJ.
- CHARNOV, E.L., R.L. LOS-DEN HARTOGH, W.T. JONES, AND J. VAN DEN ASSEM. 1981. Sex ratio evolution in a variable environment. *Nature* 289:27-33.
- DARLING, D.C., AND J.H. WERREN. Biosystematics of *Nasonia* (Hymenoptera: Pteromalidae): two new species reared from birds' nests in North America. *Ann. Entomol. Soc. Am.* in press.
- FRANK, S.A. 1985. Hierarchical selection theory and sex ratios. II. On applying the theory, and a test with fig wasps. *Evolution* 39:949-964.
- HAMILTON, W. D. 1967. Extraordinary sex ratios. *Science* 156:477-488.
- HAMILTON, W. D. 1979. Wingless and fighting males in fig wasps and other insects, pp. 167-220. In M.S. Blum and N.A. Blum (eds.), *Sexual Selection and Reproductive Competition in Insects*. Academic Press, NY.
- HERRE, E.A. 1985. Sex ratio adjustment in fig wasps. *Science* 228:896-898.
- JONES, W. T. 1982. Sex ratio and host size in a parasitoid wasp. *Behav. Ecol. Sociobiol.* 10:207-210.
- KING, B.H. 1987. Offspring sex ratios in parasitoid wasps. *Q. Rev. Biol.* 62:367-396
- KING, B.H. 1988. Sex-ratio manipulation in response to host size by the parasitoid wasp *Spalangia cameroni*: a laboratory study. *Evolution* 42:1190-1198.
- NUNNEY, L., AND R.F. LUCK. 1988. Factors influencing the optimum sex ratio in a structured population. *Theor. Popul. Biol.* 33:1-30.
- ORZACK, S.H. 1986. Sex-ratio control in a parasitic wasp, *Nasonia vitripennis*. II. Experimental analysis of an optimal sex-ratio model. *Evolution* 40:341-356.
- ORZACK, S.H., AND E.D. PARKER, JR. 1986. Sex-ratio control in a parasitic wasp, *Nasonia vitripennis*. II. Genetic variation in facultative sex-ratio adjustment. *Evolution* 40:331-340.
- SAUL, G., P.W. WHITING, S.W. SAUL, AND C.A. HEIDNER. 1965. Wildtype and

- mutant stocks of *Mormoniella*. *Genetics* 52:1317-1327.
- SKINNER, S.W. 1982. Maternally inherited sex ratio in the parasitoid wasp, *Nasonia vitripennis*. *Science* 215:1133-1134.
- SKINNER, S.W. 1983. Extrachromosomal sex ratio factors in the parasitoid wasp, *Nasonia* (=Mormoniella) *vitripennis* (Hymenoptera, Pteromalidae). Ph.D. Diss., Univ. of Utah .
- SKINNER, S.W. 1985. Son-killer--a third chromosomal factor affecting the sex ratio in the parasitoid wasp *Nasonia* (Mormoniella) *vitripennis*. *Genetics* 109:745-759.
- SKINNER, S.W., A. PAWUL, J. DUTTON, AND E. DELONG. Use of molecular probes in the study of host-range in insect-pathogen interactions. in press.
- TAYLOR, A.D. Density-dependent sex ratios: comparisons of "local mate competition" and "variation in fitness" acting separately and together. *Evolution*, in press.
- VELTHIUS, H.H., F.M. VELTHIUS-KLUPPELL, AND G.A. BOSSINK. 1965. Some aspects of the biology and population dynamics of *Nasonia vitripennis* Walker. *Entomol. Exp. Appl.* 8:205-227.
- WAAGE, J.K. 1986. Family planning in parasitoids: adaptive patterns of progeny and sex allocation, pp. 63-95. In J. Waage, and D. Greathead (eds.) *Insect Parasitoids*. Academic Press, London.
- WALKER, I. 1967. Effect of population density on the viability and fecundity in *Nasonia vitripennis* Walker (Hymenoptera, Pteromalidae). *Ecology* 48:294-301.
- WYLIE, H.G. 1965. Some factors that reduce the reproductive rate of *Nasonia vitripennis* (Walk.) at high adult population densities. *Can. Entomol.* 97:970-977.
- WYLIE, H.G. 1966. Some mechanisms that affect the sex ratio of *Nasonia vitripennis* (Walk.) (Hymenoptera: Pteromalidae) reared from superparasitized housefly pupae. *Can. Entomol.* 98:645-653.
- WERREN, J. H. 1980. Sex ratio adaptations to local mate competition in a parasitic wasp. *Science* 208:1157-1159.
- WERREN, J. H. 1983. Sex ratio evolution under local mate competition in a parasitic wasp. *Evolution* 37:116-124.

Table 1. Mean + SD sex ratio (proportion sons) and number of emerging sons and daughters per female with different numbers of females ovipositing on four hosts. (Analyzed by Kruskal-Wallis one-way ANOVA.)

<i>Nasonia giraulti</i>				
Treatment	Sons	Daughters	Sex Ratio	N
1	2.9 + 0.4	49.4 + 15.5	0.06 + 0.02	7
2	3.1 + 2.5	25.7 + 11.9	0.10 + 0.06	8
4	3.2 + 2.1	17.8 + 10.0	0.15 + 0.08	11
8	4.5 + 2.7	18.6 + 8.6	0.19 + 0.07	9
16	4.6 + 1.3	16.4 + 2.2	0.22 + 0.04	10
	H = 9.08 P = 0.06	H = 20.23 P < 0.001	H = 22.67 P < 0.001	
<i>Nasonia vitripennis</i>				
Treatment	Sons	Daughters	Sex Ratio	N
1	10.0 + 5.5	54.6 + 15.4	0.16 + 0.06	10
2	24.6 + 11.3	34.1 + 15.8	0.43 + 0.16	9
4	30.3 + 7.7	22.6 + 6.7	0.57 + 0.11	8
8	31.3 + 9.6	8.4 + 3.7	0.78 + 0.10	8
16	18.7 + 3.9	4.6 + 1.2	0.80 + 0.07	8
	H = 22.97 P < 0.001	H = 34.92 P < 0.001	H = 34.27 P < 0.001	