SEX RATIO MANIPULATION BY THE PARASITOID WASP

MUSCIDIFURAX RAPTOR IN RESPONSE TO HOST SIZE

SANDRA E. SEIDL AND BETHIA KING

Department of Biological Sciences, Northern Illinois University

DeKalb, IL 60115 USA

Under some conditions there will be selection for a female to adjust her offspring sex ratio in response to environmental variables, such as resource availability (Trivers and Willard, 1973). In parasitoid wasps, the mechanism by which females can potentially adjust their sex ratios is by controlling fertilization: unfertilized eggs develop into sons and fertilized eggs develop into daughters. Here we examine the offspring sex ratio response to resource availability, as measured by host size, in the parasitoid wasp Muscidifurax raptor by using laboratory experiments to examine the host size model (Charnov et al., 1981).

The host size model predicts that in solitary species of parasitoid wasps (one wasp develops per host) there will be selection for females that oviposit a greater proportion of daughters in large hosts than in small hosts. This prediction is based on the assumption that host size has a more positive effect on the reproductive success of daughters than of sons. A differential effect of host size on the reproductive success of males and females may occur through an effect of host size on wasp size (van den Assem, 1971; Charnov et al., 1981) or wasp development time (King, 1988).

The host size model also assumes no differential mortality of the sexes during development (Charnov et al., 1981). However, if a greater proportion of daughters emerges from large than from small hosts, females may be manipulating their sex ratios as the model predicts, or daughters may be dying in small hosts. In most parasitoid wasps, females are larger than males and so may require more resources for development (Hurlbutt, 1987).

In testing the host size model for M. raptor, we address four questions: 1) Does host size affect the sex ratio of emerging wasp offspring as predicted by the host size model; that is do a greater proportion of daughters emerge from large hosts than from

small hosts? 2) Do females preferentially oviposit daughters in large hosts or is there differential mortality of daughters in small hosts? 3) Might host size affect the reproductive success of emerging wasp offspring by affecting either wasp size or development time? 4) As assumed by the model, does host size have a greater positive effect on the reproductive success of daughters (as measured by lifetime offspring production and longevity) than of sons (as measured by male mating success and longevity)?

The host size model has been well-tested in only a few other species (Jones, 1982; King, 1988; van den Assem et al., 1989; Heinz, 1991). Further tests of the model are important because 1) the phenomenon of host size-dependent sex ratios appears to be common and widespread in parasitoid wasps (King, 1992), 2) results of previous tests of the model in other species have been mixed (see Discussion), and 3) the host size model is becoming accepted as the explanation for host size-dependent sex ratios despite very limited data on the model's assumption that host size has a greater effect on female than on male reproductive success (see Discussion).

MATERIALS AND METHODS
General Methods

A colony of <u>Muscidifurax raptor</u> was established in 1990 from wasps that emerged from <u>Musca domestica</u> pupae that had been parasitized at a poultry and sheep farm in DeKalb, Illinois. Voucher specimens of <u>M</u>. <u>raptor</u> have been deposited in the insect collection at the Illinois Natural History Survey. General maintenance of the wasps and their hosts is described in King 1988.

Two size classes of <u>M</u>. <u>domestica</u> pupae (hosts) were produced following King (1988), "small" and "large." In experiments, the "small" hosts that a female received were visibly smaller than the "large" hosts that she received. Successfully parasitized hosts were measured in the first experiment described below, and "small" hosts were significantly smaller than "large" hosts (mean $\pm$ SE = 14.63 $\pm$ 0.21 mm^3, range 7.11 - 26.37, n = 200 versus 23.65 $\pm$ 0.19 mm^3, range 15.71 - 28.84, n = 147; t = 30.7, P < 0.001). For all experiments, pupae were 1-48 h old (from the time of pupal tanning) when initially presented to the wasps. Wasps used in experiments were newly emerged (less than 2 days old), had been given a drop of honey as food, and had no previous contact with other wasps, aside from mating. Each mated female had mated with a virgin male. A few females that were observed to mate produced only sons and were excluded from analyses in the results presented here, but their exclusion did not affect conclusions. Females were presented with hosts in 4 dram glass vials with cotton plugs and a drop of honey on the side of the vial for food. Experiments were performed at 20-28°C, 24L, unless stated otherwise.

Hosts and wasps were measured as described in King 1988. Head width was used as a measure of wasp size because it is positively correlated with other measures of size, such as wing length and mass, but does not appear to be influenced by adult feeding (King, 1988).

Effects of Host Size on Wasp Offspring Sex Ratio, Size, and Development Time

To determine whether females manipulate offspring sex ratio in response to host size, 21 mated females were each given 15 small and 15 large hosts. After 24 h, each female was given a new set of hosts for another 24 h. Hosts were subsequently

isolated in gelatin capsules and checked twice daily to determine emergence time of wasp offspring. This experiment was performed at 26-28°C, 24L. Head widths of offspring from each mother's first day of hosts were measured, and number and sex ratio of offspring were determined for each female across both days of hosts.

The sex ratio of offspring from small versus large hosts was compared by a paired t-test with mother as the sampling unit. The effects of host size on wasp size and on development time were determined by analyses of covariance (ANCOVAs), with family as main effect and host volume processed concurrently as covariate.

Differential Mortality of the Sexes

To test whether there is differential mortality of daughters in small hosts during development, viability (= proportion of eggs surviving to emerge as adults) was compared between virgin and mated females (Sandlan, 1979). If there is differential mortality of daughters in small hosts, there will be greater mortality among offspring of mated females (which can produce sons and daughters) than among offspring of virgin females (which produce only sons). In this experiment, each of 17 replicates involved four females, two virgin females and two mated females. Each female was given 12 small hosts for 24 h. In each replicate, for one virgin female and for one mated female, six hosts were dissected, and the number of eggs found was multiplied by two. Offspring of the other virgin and mated females were allowed to develop to the adult stage. Offspring viability was estimated for virgin and for mated females in each replicate as number of adult offspring divided by number of eggs. (Viability values greater than one (see results) suggest that not all eggs were found; however, there is no reason to expect a difference in our ability to find eggs of virgin versus mated females.) Viability was compared between virgin and mated females by a paired t-test with replicate as the sampling unit because the females used in each replicate were the same age, mated with males of the same age, and received hosts that were similar in size and age.

Effects of Host Size on Wasp Reproductive Success

The following experiments examined how the size of host on which a wasp developed affected the wasp's subsequent reproductive success. The wasps used in the reproductive success experiments emerged from a wide range of host sizes: the largest hosts were more than three times the volume of the smallest hosts (Table 1). The host sizes used included most of the range of sizes parasitized by M. raptor in nature (Table 1). Within experiments, each replicate involved a wasp from a small host and a wasp from a large host, with the two wasps matched for age and age of mates and presented on the same day with hosts that were matched for size and age. Effects of host size on reproductive success are examined here by regressions, but conclusions were the same from paired t-tests of wasps from small versus large hosts with replicate as the sampling unit.

To determine the effects of host size on female wasp reproductive success, mated females were each provided with 15 large hosts daily until death. This experiment was designed to simulate a good environment, as hosts were large and superabundant (females never parasitized all 15 hosts). Emerged offspring were counted, and three measures of offspring production were determined for each female, lifetime offspring production, offspring production over the first two days of

life (all females lived at least this long), and peak offspring production (greatest number of offspring produced in a single day). The three measures of offspring production and days alive were each regressed on the volume of the host from which a female emerged and also on her head width.

To determine the effects of host size on male longevity, males were kept individually in 12 mm diameter X 75 mm high test tubes with cotton plugs. A drop of honey was provided as food and was wetted as needed to prevent its drying out. Each male was checked daily, and date of death was recorded. Days alive was regressed on host volume and on head width.

To determine the effects of host size on male mating success, males were each placed in a 4 dram glass vial with 10 virgin females for 4 h. Each female was then removed and placed in a 1 oz plastic vial with 5 hosts and a drop of honey for 24 h. Emerging offspring were sexed and counted. Using only females that produced at least 1 offspring, two measures of male mating success were determined for each male: the proportion of females that were inseminated (i.e., the proportion that produced any female offspring) and the number of daughters per female. Each of these measures was regressed on the volume of the host from which a male emerged and on his head width.

RESULTS
Effect of Host Size on Offspring Sex Ratio

When females were given small and large hosts simultaneously, the proportion of sons was greater from small than from large hosts (mean $\pm$ SE = 0.19 $\pm$ 0.03 versus 0.11 $\pm$ 0.02; t = 1.95; df = 20; P = 0.03) due to the production of more sons in small than in large hosts (mean $\pm$ SE = 1.62 $\pm$ 0.29 versus 0.81 $\pm$ 0.18; t = 2.19; df = 20; P = 0.04). Total number of offspring did not differ significantly between small and large hosts (t = 1.56; df = 20; P = 0.13).

Differential Mortality of the Sexes

Mated and virgin females showed no significant difference in offspring viability (mean $\pm$ SE = 1.45 $\pm$ 0.27 versus 1.27 $\pm$ 0.26; t = -0.44, df = 16, P = 0.67). Viability was not significantly different from 1.00 for either mated or virgin females (t = 1.67, df = 1, P > 0.10; t = 1.05, df = 1, P > 0.20). Thus, there was no evidence of offspring mortality.

Effect of Host Size on Wasp Size and Development Time

When potential family effects were taken into account, there were no significant effects of host size on wasp size for either females or males (females: $F_{1,97}$ = 1.55, P = 0.22; males: $F_{1,11}$ = 1.70, P = 0.22). Controlling for host size, there were no family effects on wasp size for either females or males (females: $F_{20,97}$ = 1.23, P = 0.25; males: $F_{15,11}$ = 0.69, P = 0.75). Females emerged from hosts 7.11 to 28.62 mm^3; males from hosts 8.96 to 28.84 mm^3.

When family effects were taken into account, there were no significant effects of host size on male development time ($F_{1,32}$ = 0.54, P = 0.47). Female wasps, however, took significantly longer to develop on larger hosts ($F_{1,272}$ = 8.07, P = 0.005). The difference in mean development time of daughters on large hosts minus on small hosts was calculated for each mother and averaged 0.23 days $\pm$ 0.082 SE, n = 21

mothers. When host size effects were taken into account, there were no family effects on male emergence time ($F_{18,32} = 1.04$, $P = 0.45$), but there were significant family effects on female emergence time ($F_{20,272} = 2.98$, $P = 0.000$).

<div align="center">Effect of Host Size on Wasp Reproductive Success</div>

Females did not live longer when they had developed on larger hosts or when they were larger (Tables 2 and 3). Females from larger hosts did not produce more offspring over their lifetimes, over their first two days, or on their peak day of production (Table 2). Larger females did not produce significantly more offspring than smaller females over their lifetimes or on their peak day of production but did produce more offspring over their first two days (Table 3).

Males did not live longer when they had developed on larger hosts, but they did live longer when were larger (Tables 2 and 3). Males did not have greater mating success when they had developed on larger hosts or when they were larger, regardless of whether mating success was measured as the proportion of females inseminated or as the number of daughters produced (Tables 2 and 3).

<div align="center">DISCUSSION</div>

The host size model prediction was supported. The proportion of sons produced by M. raptor was greater from small than from large hosts. This pattern is consistent with results for a southwestern U.S. strain of M. raptor (Legner, 1969) and with results for many other species of parasitoid wasps (reviewed in King, 1989, 1992).

There was no evidence of differential mortality of female M. raptor in small hosts as there was no significant difference between virgin and mated females in offspring viability. Differential mortality of the sexes could still occur within a host if a female oviposits at least one offspring of each sex in a single host. However, this seems unlikely: a female only occasionally oviposits more than one egg in a host (in 8 of 252 hosts dissected, personal observation), and in M. zaraptor, the first larva to hatch usually attacks all other eggs it encounters, and there is no evidence of differential mortality of the sexes within a host (Wylie, 1971).

The lack of evidence of differential mortality suggests that the greater proportion of sons from small versus large hosts results from maternal manipulation of sex ratio at the time of oviposition. Though host size-dependent sex ratios have been demonstrated for at least 53 species of parasitoid wasps, M. raptor is one of only 13 species in which females have been shown to manipulate sex ratio at the time of oviposition (King, 1992). In the remaining 40 species, it is not known whether sex ratio effects result from maternal manipulation or from differential mortality of the sexes.

Although the host size model prediction that a greater proportion of sons will be oviposited in small than in large hosts was supported, the model's assumption that host size has a more positive effect on the reproductive success of daughters than sons was not supported. Host size did not affect wasp size. Furthermore, there was no evidence that host size affects either female or male reproductive success.

The lack of a significant effect of host size on wasp size in this study is consistent with previous findings by Legner (1969) for a southwestern U.S. strain of M. raptor, although Legner measured wasp size by dry weight and used hosts of different sizes and age than were used in our experiments. The lack of a significant

effect of host size on <u>M</u>. <u>raptor</u> size is inconsistent with 1) Charnov et al.'s (1981) suggestion that host size differentially affects the reproductive success of male versus female wasps through a positive effect of host size on wasp size and 2) the positive relationship between host size and wasp size found in almost all other species that have been examined (references in King, 1992).

There was a statistically significant effect of host size on development time of <u>M</u>. <u>raptor</u> females, but not males, in our study. Females took about ¼ day longer to develop on larger hosts. Although statistically significant, this increase in development time was small, less than the precision of our measure, and so may not be biologically important. Furthermore, the effect of development time on fitness will be difficult to determine. Longer development time may decrease fitness if the wasp population is increasing in size or if there is high predation or parasitism of wasps while they are within their hosts; on the other hand, longer development time may increase fitness if the wasp population size is decreasing (Lewontin, 1965; Stearns, 1992). Parasitoid wasp population sizes may fluctuate considerably (ref).

Female <u>M</u>. <u>raptor</u> did not exhibit a positive relationship between host size and longevity or offspring production, regardless of how offspring production was measured. Female size was positively related to offspring production over the first two days of life, but was not related to longevity, lifetime offspring production, or peak offspring production. Positive relationships between wasp size or host size and wasp longevity or offspring production have been demonstrated in most parasitoid wasp species that have been examined (King, 1992).

Male <u>M</u>. <u>raptor</u> did not exhibit a positive relationship between host size and longevity or mating success. Male size was positively related to longevity, but not to mating success. In contrast to <u>M</u>. <u>raptor</u>, large size has been shown to increase male mating ability in some species of parasitoid wasps (van den Assem, 1976; Jones, 1982; van den Assem et al., 1989; Heinz, 1991). The effect of host size and wasp size on mating success of <u>M</u>. <u>raptor</u> males in the presence of other males has not been investigated. However, there is no obvious male-male aggression in <u>M</u>. <u>raptor</u>.

Despite statements such as, "It is widely believed that an increment of resources to a daughter leads to a greater increase in fitness (in terms of increased egg production and greater longevity for ovipositional encounters) than the same increment to a son (Charnov, 1982)" (Gauld and Bolton, 1988, p. 11) and "in parasitic Hymenoptera small hosts give rise mainly to males (Charnov et al. 1981) for in these organisms the fitness loss from being a small male is less than that of being a comparable sized female" (Southwood, 1988, p. 5), further tests of the host size model assumption of a greater effect of host size on female than on male reproductive success are needed. This assumption has been tested in only four species, in addition to <u>M</u>. <u>raptor</u>, with mixed results. In <u>Heterospilis prosopidis</u>, <u>Lariophagus distinguendus</u>, and <u>Diglyphus begini</u>, a positive effect of host size or wasp size on both male and female reproductive success has been demonstrated, and the investigators concluded that the effect is greater for females, as the host size model assumes (Jones, 1982; van den Assem et al., 1989; Heinz, 1991). In contrast, in <u>Spalangia cameroni</u> and <u>M</u>. <u>raptor</u>, the only evidence that host size may differentially affect male and female reproductive success is an effect of host size on female, but not male, development time (King, 1988; this study). However, in both species the difference in female development time on small versus large hosts was very small and

was less than the precision with which development time was measured. Also, the effect was in the opposite direction in these two species: in M. raptor, female development time increased on larger hosts; in S. cameroni, female development time decreased on larger hosts. L. distinguendus and S. cameroni are confamilials of M. raptor.

That the other investigators found a significant effect of a wasp's host size or body size on the wasp's subsequent reproductive success whereas we did not is not due to insufficient sample sizes in our study: our sample sizes were generally comparable to theirs. Rather, the magnitude of the effects in our study were less than in the other studies. For example, in our study of lifetime offspring production, host size had only a nonsignificant 1.2-fold effect, and wasp size had only a nonsignificant 2-fold effect (calculated from hosts sizes and wasp sizes in Table 1 and regression equations in Tables 2 and 3). This contrasts with a 21-fold effect of host size in Jones (1982) and a 5-fold and 25-fold effect of wasp size in van den Assem et al. (1989) and Heinz (1991), respectively. Our results suggest that there is no selection for M. raptor females to manipulate sex ratio in response to the size of M. domestica hosts; or at the least, if there is any such selection, it is weak.

Our results do not address the possibility that M. raptor's sex ratio response to host size may be adaptive in host species that are smaller or larger than M. domestica. That is, there may be a more positive effect of host size on the reproductive success of female than of male wasps in smaller (or in larger) host species and an effect in smaller (or in larger) host species may have selected for a generalized sex ratio response to host size. This possibility remains to be tested.

An alternative to the host size model for explaining M. raptor's maternal sex ratio manipulation in response to host size is that manipulation may simply be a phylogenetic holdover. Unfortunately, there is little information on sex ratio in other species of Muscidifurax. Wylie (1967) did not find a significant positive relationship between host size and offspring sex ratio for M. zaraptor. However, in his study, different host sizes were presented to different females, not simultaneously to each female; and simultaneous presentation of different host sizes provides a stronger test of the relationship (King, 1993).

LITERATURE CITED
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.
GAULD, I. AND B. BOLTON. 1988. The Hymenoptera. Oxford University Press, Oxford, UK.
HEINZ, K. M. 1991. Sex-specific reproductive consequences of body size in the solitary ectoparasitoid Diglyphus begini. Evolution 45:1511-1515.
HOLDAWAY, F. T. AND H. F. SMITH. 1932. A relation between size of host

puparia and sex ratio of <u>Alysia</u> <u>manducator</u>. Aust. J. Exp. Biol. Med. Sci. 10:247-259.

HURLBUTT, B. L. 1987. Sexual size dimorphism in parasitoid wasps. Biol. J. Linn. Soc. 30:63-89.

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 <u>Spalangia cameroni</u>: a laboratory study. Evolution 42:1190-1198.

KING, B. H. 1989. A test of local mate competition theory with a solitary species of parasitoid wasp, <u>Spalangia cameroni</u>. Oikos 54:50-54.

KING, B. H. 1991. Interspecific differences in host (Diptera: Muscidae) size and species usage among parasitoid wasps (Hymenoptera: Pteromalidae) in a poultry house. Environ. Entomol. 19:1519-1522.

KING, B. H. 1992. Sex ratio manipulation by parasitoid wasps. <u>In</u> D.L. Wrensch & M. Ebbert (eds.), Evolution and Diversity of Sex Ratio in Insects and Mites. Chapman and Hall, NY.

LEGNER, E. F. 1969. Adult emergence interval and reproduction in parasitic Hymenoptera influenced by host size and density. Ann. Entomol. Soc. Am. 62:220-226.

LEWONTIN, R. C. 1965. Selection for colonizing ability, pp. 77-91. <u>In</u> H. G. Baker and G. L. Stebbins (eds.), The Genetics of Colonizing Species. Academic Press, NY.

SANDLAN, K. P. 1979. Sex ratio regulation in <u>Coccygomimus turionella</u> Linnaeus (Hymenoptera: Ichneumonidae) and its ecological implications. Ecol. Entomol. 4:365-378.

SOUTHWOOD, T. R. E. 1988. Tactics, strategies and templets. Oikos 52:3-18.

STEARNS, S. C. 1992. The Evolution of Life Histories. Oxford University Press, Oxford, UK.

TRIVERS, R. L. AND D. E. WILLARD. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90-92.

VAN DEN ASSEM, J. 1971. Some experiments on sex ratio and sex regulation in the Pteromalid <u>Lariophagus distinguendas</u>. Neth. J. Zool. 21:373-402.

VAN DEN ASSEM, J. 1976. Male courtship behaviour, female receptivity signal, and size differences between the sexes in Pteromalinae (Hym., Chalcidoidea Pteromalidae), and comparative notes on other chalcidoids. Neth. J. Zool. 26:535-548.

VAN DEN ASSEM, J., J. J. A. VAN IERSEL, AND R. L. LOS-DEN HARTOGH. 1989. Is being large more important for female than for male parasitic wasps? Behaviour 108:160-195.

WYLIE, H. G. 1967. Some effects of host size on <u>Nasonia vitripennis</u> and <u>Muscidifurax raptor</u> (Hymenoptera: Pteromalide). Can. Entomol. 99:742-748.

WYLIE, H. G. 1971. Observations on intraspecific larval competition in three hymenopterous parasites of fly puparia. Can. Entomol. 103:137-142.

Table 1. Range of host sizes and wasp sizes used in our experiments and parasitized by Muscidifurax raptor in the field. (Sample sizes are given in Table 2.)

	Host width (mm)	Host volume (mm^3)	Wasp head width (mm)
Field study (King, 1991)	2.50 – 2.95		
Female reproductive success experiment	1.95 – 2.90	9.36 – 28.12	0.36 – 0.57
Male longevity experiment	1.70 – 2.95	6.43 – 30.1	0.39 – 0.53
Male mating success experiment	1.65 – 3.05	6.05 – 32.63	0.39 – 0.54

Table 2. Reproductive success of female and male wasps and regressions of wasp reproductive success (Y) on host volume (mm^3) (X). (Regression equations are included for heuristic purposes.)

	Mean ± SE	Range	R^2	N	P	Equation
Female Wasps:						
Longevity (days)	10.6 ± 0.7	2 – 20	0.002	39	0.40	Y = 0.03X + 10.0
Lifetime offspring production	52.1 ± 4.8	4 – 124	0.01	39	0.27	Y = 0.56X + 41.3
Number of offspring on first two days	9.7 ± 0.6	2 – 17	0.000	39	0.49	Y = 0.003X + 9.7
Number of offspring on peak day of production	9.3 ± 0.4	2 – 15	0.000	39	0.90	Y = -0.01X + 9.5
Male Wasps:						
Longevity (days)	37.0 ± 1.2	7 – 68	0.001	95	0.77	Y = -0.06X + 38.1
Daughter production per female	1.8 ± 0.07	0.5 – 2.7	0.005	60	0.60	Y = -0.005X + 1.9
Proportion of females inseminated	0.74 ± 0.03	0.20 – 1.00	0.004	60	0.62	Y = -0.002X + 0.8

Table 3. Regressions of reproductive success of female and male wasps (Y) on wasp head width (mm) (X). (Nonsignificant regression equations are included for heuristic purposes.)

	N	R^2	P	Equation
Female Wasps:				
Longevity (days)	31	0.03	0.19	Y = 13.7X + 4.0
Lifetime offspring production	31	0.06	0.09	Y = 146.8X − 20.5
Number of offspring on first two days	31	0.23	0.003	Y = 30.0X − 5.7
Number of offspring on peak day of production	31	0.06	0.09	Y = 11.7X + 3.6
Male Wasps:				
Longevity (days)	95	0.05	0.04	Y = 92.1X − 8.4
Daughter production per female	60	0.03	0.11	Y = 2.4X + 0.6
Proportion of females inseminated	60	0.02	0.12	Y = 0.8X + 0.3