

1 This is an electronic version of the article published as
2 King, B. H. 2002. Sex ratio response to conspecifics in a parasitoid wasp: test of a prediction of
3 local mate competition theory and alternative hypotheses. **Behavioural Ecology and**
4 **Sociobiology** 52:17-24. The original publication is available at www.springerlink.com.
5
6
7
8
9
10

11
12 Sex ratio response to conspecifics in a parasitoid wasp: test of a prediction of local
13 mate competition theory and alternative hypotheses
14

15
16 B. H. King
17 Department of Biological Sciences,
18 Northern Illinois University, DeKalb, IL 60115, USA
19 e-mail bking@niu.edu
20 Tel.: +1-815-7538460, Fax: +1-815-7530461
21
22
23

24 **Abstract** Maternal manipulation of offspring sex ratio in response to conspecifics
25 is considered in relation to sex ratio theory using the parasitoid wasp Spalangia
26 endius. Females produced a greater proportion of sons in response to mated but
27 not virgin females. This is the first demonstration of a differential sex ratio
28 response to virgin versus mated females and provides support for local mate
29 competition theory. More recent sex ratio models that predict sex ratio responses
30 to conspecifics, specifically constrained, perturbation, and crowding models, were
31 not supported. An increased proportion of sons in response to another mated
32 female occurred on the second day of oviposition but not on the first, and the day
33 effect resulted from experience not age. When females oviposited alone after 2 d
34 exposure to another female, they still produced a greater proportion of sons than if
35 they had always been alone, but only if the other female was mated, not if she was
36 virgin. Females do not seem to assess the presence of virgin versus mated females
37 indirectly by using a low density of males or a long latency to mate as an indicator
38 for virgin females: neither affected offspring sex ratio. That mated females
39 adjusted their sex ratios in response to other mated females but not virgin females
40 or males may be due proximally to mated females not often encountering the
41 latter. Virgin females and males are not located as deep in the oviposition
42 substrate as mated females.
43
44

45 **Key words** Sex ratio · local mate competition · parasitoid wasp · virgin

1 Introduction

2 In a wide range of taxa, mothers adjust their offspring sex ratios (proportion of
3 sons) in response to environmental factors (e.g., Charnov 1982; Dittus 1998). For
4 example, in many parasitoid wasps, mothers increase their offspring sex ratio in
5 the presence of other mothers (reviewed in King 1993, Godfray 1994). An
6 adaptive explanation for this increase was proposed by Hamilton (1967) in the
7 form of local mate competition theory (LMC). As adaptive sex ratio theories go,
8 LMC is well known and accepted (e.g., Thornhill and Alcock 1983; Krebs and
9 Davies 1993; Fellowes et al. 1999). In fact, sex ratio theory in general and LMC
10 in particular have been cited as being among the most successful areas of
11 evolutionary biology (Bull and Charnov 1988; Godfray and Hardy 1993;
12 Fellowes et al. 1999). Criteria for the success of any scientific model/theory
13 should include data other than that which led to the theory in the first place and
14 ruling out of alternative models. Both of these criteria deserve greater attention
15 and are provided here for LMC by examining sex ratio response to conspecifics
16 in the parasitoid wasp Spalangia endius in relation to LMC and to other more
17 recent adaptive sex ratio models.

18 LMC assumes that some or all mating takes place at the natal site resulting
19 in competition among brothers for mates (Hamilton 1967; Nunney and Luck
20 1988). This assumption is supported for Spalangia (Myint and Walter 1990; Hardy
21 1994). One explanation for LMC is that by increasing her proportion of sons in
22 response to other mothers, a mother increases the chance that one of her sons, and
23 not another mother's sons, inseminates the local females (Taylor 1981). In
24 addition, with the presence of other mothers, the advantage to producing daughters
25 as mates for her sons also decreases because now those daughters can become
26 mates for the other mothers' sons.

27 LMC may seem to be supported for many parasitoid wasp species (King
28 1993): consistent with LMC, overall sex ratios are often female-biased (Clausen
29 1939; Waage 1982; Luck et al. 1993); and the female-bias often decreases in the
30 presence of other females or hosts parasitized by other females (Salt 1936;
31 Jackson 1966; King 1993). However, note from the dates of these publications that
32 these empirical patterns had been demonstrated prior to, and are cited in, the first
33 LMC paper (Hamilton 1967). While additional instances of the same empirical
34 patterns are still of interest and provide greater confidence in a theory, arguably a
35 stronger test of the theory is to use an empirical pattern other than the one that led
36 to the theory in the first place (Chalmers 1982), as done here. Specifically, LMC
37 predicts that a female's sex ratio response to a virgin female should be the same as
38 when she is alone or with a male, in contrast to the increase predicted in response
39 to a mated female (equation 2, Werren 1980). The responses to virgin females and
40 to males have been little studied yet can be tested within a single species, as done
41 here.

42 Adaptive sex ratio response to conspecifics has also been described by
43 another more recent model, the constrained model, which examines the effects of
44 females constrained to produce only sons, e.g., virgins (Godfray 1990; Godfray

1 and Hardy 1993). Females may estimate the proportion of constrained females
 2 present based on their own mating status and that of conspecifics that they
 3 encounter, e.g., 0.5 for a mated female with a virgin versus 0 for a mated female
 4 alone or with another mated female. Given these estimates, in the absence of
 5 LMC, a female should produce the same sex ratio when alone as when with a
 6 mated female but should produce a decreased sex ratio when with a virgin
 7 female. The presence of LMC is expected to reduce the decrease in sex ratio seen
 8 in response to constrained females. With extreme LMC the reduction should be
 9 so great as to practically eliminate the effect of constrained females (Godfray
 10 1990). However, many parasitoid wasps, including *Spalangia*, appear to
 11 experience partial LMC, with some but not all mating occurring at the natal site
 12 (Myint and Walter 1990; Hardy 1994)). With partial LMC, the effect of
 13 constrained females is expected to be intermediate. Thus, a sex ratio decrease in
 14 response to constrained females supports the constrained model; the lack of a
 15 decrease suggests that LMC overwhelms any effect of constrained females.

16 The present study tests whether females respond directly to virgin versus
 17 mated females and whether females respond to the frequency of males or to
 18 latency to mate. Fewer males or a longer latency may indicate a greater likelihood
 19 that another female is constrained (Charnov 1982).

20 Another sex ratio model of potential relevance to *Spalangia* is the crowding
 21 model (Waage 1982; Werren 1984). The crowding model assumes: multiple
 22 females lead to crowding of offspring within hosts; crowding leads to small
 23 offspring; and being small is less detrimental to sons than daughters. Thus, this
 24 model predicts an increased proportion of sons in response to other females
 25 regardless of their mating status but not a sex ratio change in response to males.
 26 This model was designed for gregarious species. This model is less likely to apply
 27 to solitary species but is tested here for completeness because solitary species
 28 sometimes face competition for resources within a host. They occasionally
 29 produce more than one offspring per host and even more frequently oviposit
 30 multiple eggs per host (e.g., Propp and Morgan 1985; King and Seidl 1993). The
 31 crowding model is tested here by testing the assumption that offspring will be
 32 larger when from lone mothers than from pairs of mothers.

33 Finally, this study briefly examines whether differential sex ratio responses
 34 to different categories of conspecifics, such as a response to other females but not
 35 males, could be related proximally to differences in vertical distribution. If
 36 females and males spend their time at different substrate depths, females may
 37 encounter each other more often and this greater encounter rate may somehow
 38 trigger the sex ratio response, e.g., via a response to physical contact or
 39 pheromones.

40

41 **Methods**

42 The *S. endius* were from a colony established from wasps collected in 1996 from
 43 Florida, U.S.A. Experiments 1 and 3 were performed the same year, experiment 2
 44 a year later, all using a natural host, *Musca domestica*.

1 Host size was kept uniform by providing 800 mm³ of eggs with 1030 ml fly
 2 larval media (King 1988). 0-day-old hosts were presented to females in small clear
 3 plastic vials (40 mm high, 36 mm top diameter, 27 mm bottom diameter) for 24 h
 4 at about 25°C with a drop of honey on the vial wall for food. Each female had
 5 emerged from an isolated host and so had no previous contact with wasps other
 6 than her mate prior to use in an experiment.

7 Offspring sex ratio at oviposition was estimated from sex ratio at emergence
 8 because there is no evidence of differential mortality of the sexes during
 9 development in *S. endius* (Donaldson and Walter 1984; Napoleon and King 1999;
 10 King 2000). In two-female treatments, sex ratios were pooled across both females
 11 because data from such females would not be statistically independent and
 12 because genetic markers for tracking individual female's offspring have not been
 13 identified. Statistical analyses of sex ratios were on arcsine transformed values;
 14 means and 95% confidence intervals (CI) have been backtransformed. P values are
 15 two-tailed.

16 Experiment 1

17 The first experiment examined whether mated females adjust sex ratio in response
 18 to other mated females, whether they adjust sex ratio in response to males, and
 19 whether they do so on both their first and second day. This experiment also
 20 compared the physical location of females versus males in order to determine
 21 whether a lack of sex ratio response to males could be related to males not being
 22 where the females were. There were four treatments: F = one female, mated; FF =
 23 two females, mated; FM = one female and one male (not the male with whom the
 24 female had just mated), both mated; F4M = one female and four males (including
 25 the male with whom the female had just mated), all mated. In each treatment the
 26 wasp(s) were given 30 hosts for each of two successive days. Females were less
 27 than 1 d old. Sex ratios and number of offspring were analyzed separately by
 28 repeated measure analysis of variance, with day as a within-subject factor and
 29 treatment as a between-subjects factor.

30 At the end of each day the location of each wasp was recorded, as 1 if in the
 31 bottom half of the vial, 2 if in the top half. Scores were averaged across wasps of
 32 the same sex for those treatments with multiple wasps of the same sex. Within
 33 treatments, location did not differ between the first and second day for males or
 34 for females ($P > 0.25$), so days were combined prior to comparing location among
 35 treatments.

36 Experiment 2

37 The second experiment examined 1) whether, in the first experiment, the sex ratio
 38 response to conspecifics on the second day but not the first was related to age or
 39 experience, 2) whether prior exposure to other wasp(s) affected subsequent sex
 40 ratios, and 3) whether the sex ratio response to a female depended on whether she
 41 was mated or virgin.

42 Thus, this experiment was similar to the first except: 1) Females were given

1 their first set of hosts a day later. Each female spent the first day in a vial with
 2 honey. The next day, the experiment proceeded as in the first experiment, with 30
 3 hosts for each of two consecutive days. 2) The FM treatment was replaced with an
 4 FV treatment of one mated female with one virgin female. 3) After the first 2 days
 5 with hosts, each female was isolated (only one female in treatment FF) and given
 6 15 hosts for a day. Once isolated, the two females in FV could be distinguished
 7 because the virgin female produced only sons. Virgin versus mated female's sex
 8 ratios could not be distinguished when they were together; thus, treatment FV was
 9 analyzed only for the third day, when each female was isolated. 4) To test the
 10 crowding model, the head width of one offspring of each sex was measured from
 11 treatments F and FF on the third day, i.e., the treatments and day showing a sex
 12 ratio difference that could result from crowding (see Results).

13 For sex ratio, the interaction between treatment and day was examined for
 14 F, FF, F4M on days 1 and 2 and for F, FF on days 1-3. The interaction could not
 15 be examined across all four treatments on all three days because treatment F4M
 16 was not continued for the third day and the sex ratio from mated females was
 17 inseparable from that of virgin females for treatment FV on days one and two.
 18 Number of offspring per female was compared within days between treatments by
 19 t-tests, or by Mann-Whitney U when assumptions of normality or
 20 homoscedasticity were not met.

21

22 Experiment 3

23 The third experiment examined the effect of latency to mate on sex ratio. Each
 24 female mated within a day of emerging from an isolated host in the immediate
 25 mating treatment and two days later in the delayed mating treatment. Males had
 26 emerged from an isolated host within a day prior to mating. Each female was
 27 given 30 hosts for a day when she was 3 d old; this host presentation was within
 28 10 min after mating in the delayed mating treatment.

29

30 Results

31 Experiment 1

32 The effect of treatment on sex ratio depended on day (treatment-by-day
 33 interaction: $F_{3,130} = 2.98$, $P = 0.034$), so treatments were compared within each
 34 day. Specifically, each treatment was compared to treatment F because this is
 35 where LMC and the constrained model differ. On the first day of oviposition,
 36 when females were 0 d old, the offspring sex ratio produced by lone females was
 37 not significantly different from that produced by pairs of females or by females in
 38 the presence of one or four males (Table 1). In contrast, on the second day of
 39 oviposition, when females were 1 d old, the offspring sex ratio of lone females
 40 was significantly more female biased than the sex ratio of pairs of females.

41 For number of offspring per female, there was no interaction between
 42 treatment and day ($F_{3,133} = 1.16$, $P = 0.33$) and no day effect ($F_{1,133} = 0.10$, $P =$
 43 0.75), but there was a treatment effect ($F_{3,133} = 13.28$, $P < 0.001$). Comparing the
 44 daily average for each treatment to that for treatment F, the only significant effect

1 was that pairs of females produced fewer offspring per female than did lone
2 females (Table 2).

3 The biggest difference in vertical location was between males and females
4 (Table 3). Males tended to be in the top half of the vial and females in the bottom
5 half, usually on a host. Location of females did not differ significantly between
6 treatments F and FF (Mann-Whitney $U = 1453.0$, $n_1 = 52$, $n_2 = 57$, $P = 0.81$) or
7 FM and F4M (Mann-Whitney $U = 1599.0$, $n_1 = 57$, $n_2 = 58$, $P = 0.59$). In the
8 presence of males, females were slightly but not significantly lower in the vial (F
9 and FF versus FM and F4M: Mann-Whitney $U = 5720.5$, $P = 0.08$).

10

11 Experiment 2

12 The effect of treatment on sex ratio depended on day (treatment-by-day
13 interactions for F, FF, F4M on days 1 and 2: $F_{2, 85} = 4.43$, $P = 0.015$; and for F, FF
14 on days 1-3: $F_{2, 56} = 4.44$, $P = 0.016$), so again treatments were compared within
15 each day. On the first day of oviposition, when females were 1 d old, sex ratios of
16 lone females were not significantly different from those of pairs of mated females
17 or mated females with males (Table 4). On the second day of oviposition, when
18 females were 2 d old, sex ratios of lone mated females were significantly more
19 female-biased than those of pairs of females, although still not significantly
20 different from those of mated females with males.

21 On both the first and second day of oviposition, number of offspring per
22 female was significantly lower for pairs of females than for lone females,
23 regardless of whether the pairs were both mated or one mated and one virgin
24 (Table 5). Number of offspring per female did not differ significantly between the
25 mated-mated and the virgin-mated treatment. Number of offspring per female was
26 not significantly different when a female was alone versus with males.

27 On the third day of oviposition, females in all treatments were alone. Sex
28 ratio from the lone female treatment was still more female-biased than from the
29 paired mated-females treatment and was not different from that of mated females
30 who had been with virgin females previously (Table 4). Number of offspring did
31 not differ significantly among treatments (Table 5).

32

33 Experiment 3

34 Proportion of sons was not significantly greater for females that mated
35 immediately than females that mated 2 d later (mean = 0.15, 95% CI = 0.10 - 0.20,
36 $n = 41$ versus mean = 0.19, 95% CI = 0.12 - 0.28, $n = 41$; $t_{80} = 1.12$, $P = 0.27$).
37 Likewise, number of offspring did not differ significantly between females that
38 mated within a day of emergence versus two days later (mean \pm s.e. = 12.5 ± 0.6 ,
39 $n = 41$ versus 12.1 ± 0.6 , $n = 41$; $t = 0.52$, $df = 40$, $P = 0.61$).

40

41 Discussion

42 Testing sex ratio theory

43 S. endius's sex ratio response to conspecifics is better explained by LMC than by
44 the constrained, crowding and perturbation models. S. endius's production of a

1 greater proportion of sons in response to mated females but not virgin females or
 2 males was consistent with effects of LMC but not with effects of constrained
 3 females or crowding. LMC was supported even though *Spalangia* experiences just
 4 partial LMC and is a solitary species (one offspring per host). LMC was originally
 5 designed for gregarious species (multiple offspring per host). However, when
 6 solitary species have clumped hosts, as is true for *Spalangia* (personal
 7 observation), they are described as quasigregarious and LMC should apply (van
 8 den Assem et al. 1980). In fact, LMC's prediction of a greater proportion of sons
 9 in response to other mothers has actually been reported for a greater proportion of
 10 solitary than gregarious species (King 1993).

11 LMC's prediction of a differential sex ratio response to virgin versus mated
 12 females assumes that virgins oviposit in natural populations. In most parasitoid
 13 wasps including *S. endius*, virgin females do oviposit, often as much as mated
 14 females (reviewed in Godfray, 1994; King in press); and virgins have been
 15 collected in natural populations, although their prevalence is often low (Godfray
 16 and Hardy 1993).

17 I have not discussed numerical values of sex ratio despite the sex ratio of
 18 0.21 - 0.23 observed with two mothers (Tables 1, 3) being close to the 0.25
 19 predicted by Hamilton's (1979) LMC model. (That the value with one mother is
 20 greater than the predicted 0% would traditionally be explained by the suggestion
 21 that a lone female should produce just enough sons to inseminate her own
 22 daughters (Hamilton 1967).) My focus has instead been on sex ratio pattern,
 23 specifically an increase in proportion of sons with increasing mothers, because
 24 this pattern appears to be quite robust. It is met with asynchronous or synchronous
 25 oviposition and with or without postdispersal mating by males (Nunney and Luck
 26 1988). In contrast, predictions of the sex ratio value expected at any given number
 27 of mothers is highly dependent on these parameters and others (Bull and Charnov
 28 1988), many of which are difficult to estimate with much confidence.

29 The crowding model was not supported in *S. endius* because the model
 30 assumes that offspring size is affected by another ovipositing female's presence,
 31 but in *S. endius* it was not.

32 The perturbation model, another natural selection model that predicts a sex
 33 ratio response to conspecifics (Werren and Charnov 1978), also was not supported
 34 in *S. endius*. The model assumes overlapping generations, which *S. endius* lacks:
 35 development duration in *S. endius* is at least twice as long as adult longevity
 36 (personal observation; Napoleon and King 1999).

37 Attempts to distinguish among all of these natural selection sex ratio models
 38 in a single species have been rare (but see King 1996), partly because the
 39 constrained model is relatively recent. Some earlier studies have distinguished
 40 between LMC and the crowding models (e.g., Werren 1980; King 1992).

41 Although the sex ratio response to mated females has been examined in
 42 dozens of species (e.g., King 1993), the response to virgin versus mated females
 43 has been examined in only two other species besides *S. endius*, *Nasonia*
 44 *vitripennis* and *Bracon hebetor* (Werren 1984; Ode et al. 1997). Both differ from

1 S. endius in that females adjust their sex ratio in the presence of another mated
 2 female but also in the presence of a virgin female, although the pattern in B.
 3 hebetor could be an artifact of experimental design (see "Proximal Mechanisms"
 4 below).

5 This lack of support for LMC in terms of the response to virgin females, is
 6 of special interest in N. vitripennis because it is probably the best known, most
 7 frequently cited species in relation to LMC (e.g., Thornhill and Alcock 1983;
 8 Krebs and Davies 1993). In N. vitripennis, both mated and constrained (sperm-
 9 lacking) females are found at oviposition sites (Skinner 1983). If LMC applies,
 10 there should be selection against a general response to conspecifics as the
 11 mechanism for responding to mated females because according to LMC, selection
 12 will favor different sex ratio responses to mated versus constrained females (Table
 13 3). A differential sex ratio response to mated versus constrained females in N.
 14 vitripennis is still possible. Perhaps females respond to a trait that covaries with
 15 the lack of sperm in nature but not in Werren's (1984) laboratory experiment.

16 In addition to support for LMC from intraspecific tests, interspecific tests
 17 also provide some support. In Nasonia the greatest proportion of sons occurs in the
 18 congener with the highest rate of within host mating, consistent with LMC
 19 (Drapeau and Werren 1999), although based on just three species (all yet known
 20 in Nasonia). A recent study of 44 species of non-pollinating fig wasps provides
 21 some support for LMC and, in contrast to earlier interspecific tests, controls for
 22 phylogeny (Fellowes et al. 1999).

23 Support for the constrained model is very limited. A small proportion of
 24 parasitoid wasp species lack an increase in sex ratio in the presence of other
 25 mothers (reviewed in King 1993), consistent with the constrained model. If the
 26 constrained model is responsible for this pattern, these species should also exhibit
 27 a decreased proportion of sons in response to constrained females relative to
 28 mated and lone females, something not yet examined. However, as noted above,
 29 none of the three species for which sex ratio response to constrained females has
 30 been examined exhibit such a decreased proportion of sons (Werren 1984; Ode et
 31 al. 1997; this study).

32 Ode et al. (1997) suggest that female-biased sex ratios in the parasitoid
 33 wasp B. hebetor have been selected for due to constrained females in the
 34 population. However, the B. hebetor females studied by Ode et al. (1997)
 35 increased the proportion of sons in response to other mated mothers, and this
 36 pattern is not consistent with the constrained model. This pattern is consistent
 37 with LMC theory. Ode et al. (1997) suggest ruling out LMC based on the
 38 observation of outbreeding in B. hebetor. However, in the absence of inbreeding,
 39 competition among brothers for mates is sufficient to select for LMC sex ratios
 40 (Taylor 1981). Competition among brothers has been neither demonstrated nor
 41 ruled out for B. hebetor. Current data on B. hebetor's sex ratio response to virgin
 42 females do not support either the constrained model or LMC but with a more
 43 natural experimental design might support LMC (see below). The constrained
 44 model has received some empirical support from a thrips (e.g., Kranz et al. 2000).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

Proximal Mechanisms

The prediction of a different sex ratio response to virgin versus mated females depends on the assumption that mated females can obtain information about the presence of virgin versus mated females. However, a different response to virgin versus mated females does not require that females distinguish between them when presented with both. Encountering virgin females less frequently than mated females despite their presence in the population is a perfectly valid proximal mechanism for behaving differently to them. This provides a particularly straightforward mechanism by which the proper LMC response may be achieved since the proper LMC sex ratio response to a virgin female is the same as when alone. An analogy would be that selection can favor avoiding inbreeding but this does not have to be by recognizing kin from nonkin; rather, one sex may simply disperse and hence not be encountered.

Differences in encounter rate due to differences in spatial distribution may be the proximal mechanism for the differences in sex ratio response of *S. endius* to mated versus virgin females and mated females versus males. A female is probably more likely to encounter another female than a male given that males were found significantly higher in their containers than females. Virgin females are also found higher than mated females when hosts are present (King in press). The gender differences in spatial distribution of *S. endius* in the laboratory are corroborated by those of *S. cameroni* in the field (Donaldson and Walter 1984); laboratory gender differences of *B. hebetor* are also corroborated by field data (Guertin et al. 1996). Number of interactions between females in *N. vitripennis* correlates with sex ratio under some conditions (King et al. 1995 and references therein). The effect of interactions does not appear to be related to simple physical contact in *N. vitripennis* (King et al. 1995) or in *S. cameroni* (King 1996). Encounter rate may play a role in the proximal mechanisms of a wide range of behaviors (e.g., Uvarov 1966; Gordon 1994).

The potential importance of encounter rate suggests that response to virgin versus mated females may be worth revisiting in *B. hebetor*. That females responded to mated and virgin females with the same sex ratio, contrary to LMC, could be an artifact of the small depth of the dishes used (Ode et al. 1997). When given the opportunity, virgin *B. hebetor* females spend more time near the surface than mated females do (Guertin et al. 1996). Thus, with a deeper container more similar to their natural habitat, mated females should encounter mated females more than virgin females; in which case mated but not virgin females might evoke a sex ratio response. (The depth difference observed in the laboratory was not corroborated by field data, but the field study may have wiped out any differences by combining the top 5 cm (Ode et al. 1997).) This concern about container size probably does not explain *N. vitripennis*'s lack of a differential sex ratio response to virgin versus mated females because deeper containers were used (Werren 1984).

1 Besides obtaining information about virgin versus mated females by
 2 differences in encounter rate as just suggested, behavioral and pheromonal
 3 differences may also be used. Such differences have been documented in
 4 parasitoid wasps (e.g., Fauvergue et al. 1995; McNeil and Brodeur, 1995;
 5 Pompanon 1995) and other insects (e.g., Andersson et al. 2000; Ayasse et al.
 6 1999; Tompkins and Hall 1981; Belmain et al. 2000).

7 Whatever the proximal mechanism of the different sex ratio responses by S.
 8 endius, the effects are not immediate. There was a sex ratio response on the
 9 second but not the first day of oviposition, and the effect persisted even after
 10 females were alone.

11 Being in the presence of another mated female versus alone caused females
 12 to produce a greater proportion of sons on their second day of oviposition but not
 13 on their first. Amount of experience and not maternal age determined whether a
 14 female manipulated sex ratio in response to another mated female: a sex ratio
 15 response occurred on the second day of being with another female in both
 16 experiments one and two despite females being 1 d older in experiment two. The
 17 sex ratio response to another mated female persisted for a day after exposure.

18 Effects of experience on sex ratio response to other females have not been
 19 well explored in other species. In the mite Typhlodromus occidentalis, single
 20 females produce a greater proportion of daughters than do sets of ten and four
 21 females respectively but, similar to S. endius, not on the first day of oviposition,
 22 only from days two to seven (Nagelkerke and Sabelis 1998).

23 Besides not responding to direct encounters with virgin females, S. endius
 24 females also did not respond indirectly. Specifically, they did not seem to assess
 25 the potential of encountering mated versus virgin females by how many males
 26 they encountered or by latency to mate: neither significantly affected sex ratio.
 27 Comparison of S. endius to other parasitoid wasp species reveals a diversity of
 28 responses. Like S. endius, S. cameroni's offspring sex ratio is unaffected by
 29 latency to mate (B.H. King, unpublished); however, in three other species a
 30 greater latency results in a greater proportion of daughters (Hoelscher and Vinson
 31 1971; Rotary and Gerling 1973; Fauvergue et al. 1998). B. hebetor's sex ratio is
 32 unaffected by exposure to a conspecific male (Ode et al. 1997). In N. vitripennis,
 33 sex ratio is affected by exposure to three but not one or two conspecific male(s)
 34 (Wylie 1966, 1976). Brachymeria intermedia generally decreases, not increases,
 35 the proportion of sons it produces when in the presence of an increasing
 36 proportion of adult males (Mohamed and Coppel 1986).

37
 38
 39
 40
 41 **Acknowledgements** Thanks to C. Geden for providing starter S. endius, R.
 42 Anderson and L. Baroody for counting wasps, and R. King for comments on this
 43 manuscript. This research was supported by Northern Illinois University's
 44 Department of Biological Sciences and complies with U.S. laws.

References

- 1
2 Andersson J, Borg-Karlson AK, Wiklund C (2000) Sexual cooperation and
3 conflict in butterflies: a male-transferred anti-aphrodisiac reduces harassment
4 of recently mated females. *Proc Roy Soc London B* 267:1271--1275
- 5 Assem J van den, Gijswijt MJ, Nubel BK (1980) Observations on courtship and
6 mating strategies in a few species of parasitic wasps (Chalcidoidea). *Neth J*
7 *Zool* 30:208--227
- 8 Ayasse M, Engels W, Lubke G, Taghizadeh T, Francke W (1999) Mating
9 expenditures reduced via female sex pheromone modulation in the primitively
10 eusocial halictine bee, *Lasioglossum (Evylaeus) malachurum* (Hymenoptera :
11 Halictidae). *Behav Ecol Sociobiol* 45:95--106
- 12 Belmain SR, Simmonds MSJ, Blaney WM (2000) Behavioral responses of adult
13 deathwatch beetles, *Xestobium rufovillosum* de Geer (Coleoptera: Anobiidae),
14 to light and dark. *J Ins Behav* 13:15--26
- 15 Bull JJ, Charnov EL (1988) How fundamental are Fisherian sex ratios? *Oxford*
16 *Surveys Evol Biol* 5:96--135
- 17 Chalmers, AF (1982) What is this thing called science? University of Queensland
18 Press, St. Lucia
- 19 Charnov EL (1982) The theory of sex allocation. Princeton University Press,
20 Princeton, NJ
- 21 Clausen CD (1939) The effect of host size upon the sex ratio of hymenopterous
22 parasites and its relation to methods of rearing and colonization. *J N Y*
23 *Entomol Soc* 47:1--9
- 24 Dittus WPJ (1998) Birth sex ratios in toque macaques and other mammals:
25 integrating the effects of maternal condition and competition. *Behav Ecol*
26 *Sociobiol* 44:149--160
- 27 Donaldson JS, Walter GH (1984) Sex ratios of *Spalangia endius*
28 (Hymenoptera:Pteromalidae), in relation to current theory. *Ecol Entomol*
29 9:395--402
- 30 Drapeau MD, Werren JH (1999) Differences in mating behavior and sex ratio
31 between three sibling species of *Nasonia*. *Evol Ecol Res* 1:223--234
- 32 Fauvergue X, Hopper KR, Antolin MF (1995) Mate finding via a trail sex
33 pheromone by a parasitoid wasp. *Proc Natl Acad Sci USA* 92:900--904
- 34 Fauvergue X, Hopper KR, Antolin MF, Kazmer DJ (1998) Does time until mating
35 affect progeny sex ratio - a manipulative experiment with the parasitoid wasp
36 *Aphelinus asychis*. *J Evol Biol* 11:611--622
- 37 Fellowes MDE, Compton SG, Cook JM (1999) Sex allocation and local mate
38 competition in Old World non-pollinating fig wasps. *Behav Ecol Sociobiol*
39 46:95--102
- 40 Godfray HCJ (1990) The causes and consequences of constrained sex allocation in
41 haplodiploid animals. *J Evol Biol* 3:3--17
- 42 Godfray HCJ (1994) Parasitoids. Princeton University Press, Princeton, NJ
- 43 Godfray HCJ, Hardy ICW (1993) Sex ratio and virginity in haplodiploid insects.
44 In: Wrensch DL, Ebbert M (eds) Evolution and diversity of sex ratio in insects

- 1 and mites. Chapman and Hall, New York, NY, pp 402--417
- 2 Gordon DM (1994) How social insect colonies respond to variable environments.
3 In: Leslie RA (ed) Behavioral mechanisms in evolutionary ecology. University
4 of Chicago Press, Chicago, IL, pp 409--422
- 5 Guertin DS, Ode PJ, Strand MR, Antolin MF (1996) Host-searching and mating in
6 an outbreeding parasitoid wasp. *Ecol Entomol* 21:27--33
- 7 Hamilton WD (1967) Extraordinary sex ratios. *Science* 156:477--488
- 8 Hamilton WD (1979) Wingless and fighting males in fig wasps and other insects.
9 In: Blum MS, Blum NA (eds) Sexual selection and reproductive competition
10 in insects. Academic Press, New York, NY, pp 167--220
- 11 Hardy ICW (1994) Sex ratio and mating structure in the parasitoid Hymenoptera.
12 *Oikos* 69:3--20
- 13 Hoelscher CE, Vinson SB (1971) The sex ratio of a hymenopterous parasitoid,
14 *Campoletis perdinctus*, as affected by photoperiod, mating, and temperature.
15 *Ann Entomol Soc* 64:1373--1376
- 16 Jackson DJ (1966) Observations on the biology of the *Caraphractus cinctus*
17 Walker (Hymenoptera: Mymaridae), a parasite of the eggs of Dytiscidae. III
18 The adult life and sex ratio. *Trans R Entomol Soc Lond* 118:23--49
- 19 King BH (1988) Sex-ratio manipulation in response to host size by the parasitoid
20 wasp *Spalangia cameroni*: a laboratory study. *Evolution* 42:1190--1198
- 21 King BH (1992) Sex ratios of the wasp *Nasonia vitripennis* from self- versus
22 conspecifically-parasitized hosts: local mate competition versus host quality
23 models. *J Evol Biol* 5:445--455
- 24 King BH (1993) Sex ratio manipulation by parasitoid wasps. In: Wrensch DL,
25 Ebbert M (eds) Evolution and diversity of sex ratio in insects and mites.
26 Chapman and Hall, New York, NY, pp 418--441
- 27 King BH (1996) Sex ratio responses to other parasitoid wasps: multiple adaptive
28 explanations. *Behav Ecol Sociobiol* 39:367--374
- 29 King BH (2000) Sex ratio and oviposition responses to host age and the fitness
30 consequences to mother and offspring in the parasitoid wasp *Spalangia endius*.
31 *Behav Ecol Sociobiol* 48:316--320
- 32 King BH (in press) Breeding strategies in females of the parasitoid wasp
33 *Spalangia endius*: effects of mating status and size. *J Ins Behav*
- 34 King BH, Crowe ML, Skinner SW (1995) Effect of host density on offspring sex
35 ratios and behavioral interactions between females in the parasitoid wasp *Nasonia*
36 *vitripennis* (Hymenoptera: Pteromalidae). *J Ins Behav* 8:89--102
- 37 King BH, Seidl SE (1993) Sex ratio response of the parasitoid wasp *Muscidifurax*
38 *raptor* to other females. *Oecologia* 94:428--433
- 39 Kranz BD, Schwarz MP, Giles LC, Crespi BJ (2000) Split sex ratios and virginity
40 in a gall-inducing thrips. *J Evol Biol* 13:700--706
- 41 Krebs JR, Davies NB (1993) An introduction to behavioral ecology, 3rd ed.
42 Blackwell Scientific, Oxford
- 43 Luck RF, Stouthamer R, Nunney LP (1993) Sex determination and sex ratio
44 patterns in parasitic Hymenoptera. In: Wrensch DL, Ebbert M (eds) Evolution

- 1 and diversity of sex ratio in insects and mites. Chapman and Hall, New York,
 2 NY, pp 442--476
- 3 McNeil JN, Brodeur J (1995) Pheromone-mediated mating in the aphid parasitoid,
 4 Aphidius nigripes (Hymenoptera: Aphididae). J Chem Ecol 21:959--972
- 5 Mohamed MA, Coppel HC (1986) Sex ratio regulation in Brachymeria intermedia, a
 6 pupal gypsy moth parasitoid. Can J Zool 64:1412--1415
- 7 Myint WW, Walter GH (1990) Behaviour of Spalangia cameroni males and sex ratio
 8 theory. Oikos 59:163--174
- 9 Nagelkerke CJ, Sabelis MW (1998) Precise control of sex allocation in pseudo-
 10 arrhenotokous phytoseiid mites. J Evol Biol 11:649--684
- 11 Napoleon ME, King BH (1999) Offspring sex ratio response to host size in the
 12 parasitoid wasp Spalangia endius. Behav Ecol Sociobiol 46:325--332
- 13 Nunney L, Luck RF (1988) Factors influencing the optimum sex ratio in a structured
 14 population. Theor Popul Biol 33:1--30
- 15 Ode PJ, Antolin MF, Strand MR (1997) Constrained oviposition and female-biased
 16 sex allocation in a parasitic wasp. Oecologia 109:547--555
- 17 Pompanon F, Fouillet P, Bouletreau M (1995) Emergence rhythms and protandry in
 18 relation to daily patterns of locomotor activity in Trichogramma species. Evol
 19 Ecol 9:467--477
- 20 Propp GD, Morgan PB (1985) Effect of host distribution on parasitoidism of
 21 house-fly (Diptera: Muscidae) pupae by Spalangia spp and Muscidifurax
 22 raptor (Hymenoptera: Pteromalidae). Can Entomol 117:515--524
- 23 Rotary N, Gerling D (1973) The influence of some external factors upon the sex ratio
 24 of Bracon hebetor Say (Hymenoptera: Braconidae). Environ Entomol 2:134--138
- 25 Salt G (1936) Experimental studies in insect parasitism. IV. The effect of
 26 superparasitism on populations of Trichogramma evanescens. J Exp Biol
 27 13:363--375
- 28 Skinner SW (1983) Extrachromosomal sex ratio factors in the parasitoid wasp,
 29 Nasonia (= Mormoniella) vitripennis (Hymenoptera, Pteromalidae). PhD
 30 Dissertation, University of Utah
- 31 Taylor PD (1981) Intra-sex and inter-sex sibling interactions as sex ratio determinants.
 32 Nature 291: 64-66
- 33 Thornhill R, Alcock J (1983) The evolution of insect mating systems. Harvard
 34 University Press, Cambridge, MA
- 35 Tompkins L, Hall J (1981) The different effects on courtship of volatile compounds
 36 from mated and virgin Drosophila females. J Ins Physiol 27:17--21
- 37 Uvarov B (1966) Grasshoppers and locusts, vol I. Cambridge University Press,
 38 London
- 39 Waage JK (1982) Sex ratio and population dynamics of natural enemies--some
 40 possible interactions. Ann Appl Biol 101:159--164
- 41 Werren JH (1980) Sex ratio adaptations to local mate competition in a parasitic wasp.
 42 Science 208:1157--1159
- 43 Werren JH (1984) Brood size and sex ratio regulation in the parasitic wasp Nasonia
 44 vitripennis (Walker) (Hymenoptera: Pteromalidae). Neth J Zool 34:123--143

- 1 Werren JH, Charnov EL (1978) Facultative sex ratios and population dynamics.
 2 Nature 272:349--350
 3 Wylie HG (1966) Some mechanisms that affect the sex ratio of Nasonia vitripennis
 4 (Walk.) (Hymenoptera: Pteromalidae) reared from superparasitized housefly
 5 pupae. Can Entomol 98:645--653
 6 Wylie HG (1976) Observations on life history and sex ratio variability of
 7 Eupteromalus dubius (Hymenoptera: Pteromalidae), a parasite of
 8 cyclorrhaphous Diptera. Can Entomol 108:1267—1274
 9
 10
 11
 12
 13

14 **Table 1** Proportion of sons on days 1 and 2 of oviposition in experiment 1. F =
 15 mated female, M = mated male, 4M = 4 mated males
 16

	Day 1 0 d old Mother			Day 2 1 d old Mother		
	Mean	95% CI	(n)	Mean	95% CI	(n)
F	0.17	0.13 - 0.21	(36)	0.10	0.067 - 0.14	(36)
FF	0.18	0.15 - 0.21	(37)	0.21	0.16 - 0.26	(36)
FM	0.15	0.11 - 0.19	(37)	0.11	0.067 - 0.15	(36)
F4M	0.21	0.16 - 0.26	(28)	0.15	0.11 - 0.20	(27)
F vs FF	$t_{71} = 0.38, P = 0.71$			$t_{70} = 3.62, P = 0.001$		
F vs FM	$t_{71} = 0.83, P = 0.41$			$t_{70} = 0.25, P = 0.81$		
F vs F4M	$t_{62} = 1.29, P = 0.20$			$t_{61} = 1.92, P = 0.06$		

31

Table 2 Number of offspring per female on days 1 and 2 of oviposition in experiment 1. F = mated female, M = mated male, 4M = 4 mated males

	Day 1 0 d old Mother		Day 2 1 d old Mother	
	Mean \pm s.e.	n	Mean \pm s.e.	n
F	13.8 \pm 0.89	37	11.7 \pm 0.68	36
FF	8.6 \pm 0.47	37	8.9 \pm 0.42	36
FM	12.9 \pm 0.95	37	13.4 \pm 0.75	36
F4M	12.4 \pm 1.12	30	12.3 \pm 0.87	29

Comparisons for day 1 and 2 averages:

F vs FF	$t_{70} = 6.68, P < 0.001$
F vs FM	$t_{70} = 0.57, P = 0.57$
F vs F4M	$t_{63} = 0.39, P = 0.70$

Table 3 Average location of female wasps and male wasps in experiment 1. F = mated female, M = mated male, 4M = 4 mated males

	bottom <-----> top of vial				
score:	1	1.25	1.5	1.75	2
Females:					
F	42				10
FF	43		10		4
FM	51				6
F4M	50				8
Males:					
FM	9				47
F4M		1	10	16	29

Table 4 Observed proportion of sons from all females on days 1 and 2 of oviposition and from isolated mated female on day 3 in experiment 2. F = mated female, M = mated male, 4M = 4 mated males, V = virgin female

	Day 1 = 1 d old Mother			Day 2 = 2 d old Mother			Day 3 = 3 d old Mother		
	Mean	n	95% CI	Mean	n	95% CI	Mean	n	95% CI
F	0.14	30	0.094 - 0.19	0.09	30	0.047 - 0.15	0.12	39	0.064 - 0.20
FF	0.16	30	0.12 - 0.20	0.22	30	0.18 - 0.27	0.23	39	0.18 - 0.28
FV	0.46	29	0.39 - 0.53	0.48	29	0.41 - 0.55	0.13	34	0.070 - 0.20
F4M	0.15	28	0.11 - 0.20	0.12	28	0.080 - 0.18			
F vs FF	$t_{58} = 0.66, P = 0.51$			$t_{58} = 3.55, P = 0.001$			$t_{76} = 2.38, P = 0.020$		
F vs FV							$t_{71} = 0.06, P = 0.95$		
F vs F4M	$t_{56} = 0.48, P = 0.63$			$t_{56} = 0.36, P = 0.92$					

Table 5 Number of offspring per female on days 1 and 2 of oviposition and from isolated mated female on day 3 in experiment 2. F = mated female, M = mated male, 4M = 4 mated males, V = virgin female

	Day 1 = 1 d old Mother		Day 2 = 2 d old Mother		Day 3 = 3 d old Mother	
	Mean \pm s.e.	n	Mean \pm s.e.	n	Mean \pm s.e.	n
F	14.0 \pm 1.06	30	11.5 \pm 0.80	30	9.7 \pm 0.46	40
FF	10.4 \pm 0.46	30	8.6 \pm 0.36	30	9.9 \pm 0.37	39
FV	9.5 \pm 0.52	29	8.1 \pm 0.43	29	9.2 \pm 0.43	34
F4M	15.4 \pm 0.86	28	12.0 \pm 0.65	28		
F vs FF	U = 264.5, P = 0.006		U = 239.0, P = 0.002		t ₇₇ = 0.37, P = 0.71	
F vs FV	U = 213.0, P = 0.0007		t ₅₇ = 3.65, P = 0.001		t ₇₂ = 0.69, P = 0.50	
FV vs FF	t ₅₇ = 1.37, P = 0.18		U = 400.0, P = 0.59			
F vs F4M	t ₅₆ = 1.07, P = 0.29		t ₅₆ = 0.55, P = 0.59			