

Genetic Variation for Sex Ratio Traits Within a Natural Population of a Parasitic Wasp, *Nasonia vitripennis*

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ABSTRACT

By analyzing isofemale strains extracted from a natural population of *Nasonia vitripennis*, we detected variation for the sex ratios produced in fresh hosts (first sex ratios) and in previously parasitized hosts (second sex ratios). Under simple assumptions of population structure, this between-strain heterogeneity of first sex ratios results in heterogeneity of fitnesses. There is approximately ten percent difference in average fitnesses between the strains. (The fitnesses of second sex ratios are analyzed in the accompanying paper.) Average first and average second sex ratios are uncorrelated. There is significant between-female heterogeneity within some strains for first sex ratios but not for second sex ratios. In addition, the average direct-developing and diapause first sex ratios (but not second sex ratios) are significantly correlated. There are significant correlations between the direct-developing and diapause sex ratios produced by the same female. The strains differ in their effects on the sex ratio and size of another female's brood in the same host. Data on these types of variation for sex ratio traits are essential for further progress in the study of sex ratio evolution.

A major challenge in the study of sex ratio evolution is reconciling theory and data. The nature of sex ratio theory makes this reconciliation especially important. Many attempts at reconciling theory and data in evolutionary biology have foundered because of an imprecise understanding of the mapping between fitness and phenotype. In contrast, a virtue of our theories of sex ratio evolution is that there is less ambiguity than usual as to the nature of this mapping.

Several issues need to be addressed as part of this reconciliation. First, our knowledge of variation for sex ratio traits in natural populations is inadequate. Second, there is uncertainty in some instances as to how sex ratio theory should be tested (ORZACK 1990a). Third, there is ambiguity as to the interpretation of some patterns [as noted by KARLIN and LESSARD (1986) and others]. For example, an even primary sex ratio in a species with chromosomal sex determination can be regarded as the result of the frequency-dependent selection process described by FISHER (1930) or as a result of the constraining nature of chromosomal segregation.

This paper is a contribution to our empirical understanding. We have previously documented variation for sex ratio traits (PARKER and ORZACK 1985; ORZACK and PARKER 1986; ORZACK 1986) among

laboratory strains of a parasitic wasp, *Nasonia vitripennis* (Pteromalidae). Here we describe variation for sex ratio traits among isofemale strains extracted from a natural population. To motivate our analysis more clearly we first present a brief discussion of the underlying evolutionary framework.

We measured two sex ratio traits. The first is the sex ratio produced by a female in an unparasitized host (or hosts). The second is the sex ratio produced by a female when presented with a parasitized host. Following the convention of ORZACK and PARKER (1986), the former trait is denoted a "first sex ratio" and the latter a "second sex ratio." There are several reasons for this choice of traits. The first is their simplicity. It is easy to measure these characters for many females. Second, the distinction between the two encapsulates the qualitative difference between two situations thought to be distinct in an evolutionary sense. Under conditions of local mate competition (HAMILTON 1967; TAYLOR and BULMER 1980) the optimal first sex ratio is female-biased but to an extent dependent upon the number of females contributing offspring to the mating pool. All females are assumed to have the same information about the number of other females present. Our experimental condition of one female is extreme but there is no ambiguity about the information she possesses relative to other females and it mimics what may be a common situation in nature.

The choice of the second sex ratio also was motivated by a theoretical result relating to local mate

This paper is dedicated to the memory of our friend and teacher, ERNST CASPARI.

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competition: when a female encounters a previously parasitized host, she should produce a sex ratio distinct from her first sex ratio. In this situation the two females have *different* information about the number of other females present. Hence, a weak test of the theory is to determine whether first and second sex ratios are different. In addition, there is a quantitative prediction for the second sex ratio (SUZUKI and IWASA 1980; WERREN 1980) which one can test (ORZACK 1986, 1990a).

The analysis of genetic variation *within* populations is of special significance since it is without some complications that can hinder other investigations. Differences between populations in their level of inbreeding can affect the evolution of first sex ratios (HERRE 1985) as might differences between populations in their initial genetic composition or in their ages. In the case of second sex ratios it is difficult to predict the consequences of such effects because there is no dynamical model of their evolution.

MATERIALS AND METHODS

In July 1986 parasitized fly pupae were collected from bird nests near Södertälje, a city approximately 20 km WSW of Stockholm, Sweden. We chose a single mated female from each nest to establish a strain. Hence, each strain originates from a different nest. The strains are designated as *MS1*, *MS23*, *MS33*, *MS37*, *MS43*, *MS51*, *MS56*, *MS58*, *MS67*, *MS71*, *MS82*, and *MS92*. (In the figures we refer to these strains without using the *MS* prefix.)

In Chicago, one generation prior to measurement of first and second sex ratios (in August 1987 and January 1988), the strains were reconstituted from diapause larvae. (The larval diapause allows one to maintain most strains in "limbo" for up to 1 year or so. This circumvents some of the possible effects of continued culture on sex ratio behaviors.) These larvae had been collected a generation after arrival of the strains there in March 1987. Prior to this time, save for two to five generations of live culture, all strains except *MS23* and *MS58* were maintained in diapause in Lake Charles. *MS23* and *MS58* were maintained via live culture (approximately 16 generations).

In Lake Charles, first sex ratios were measured in February 1987 and May-July 1987. In the former case, the strains had experienced two to five generations of live culture. In the latter, the strains had experienced from eight to 12 generations of live culture.

The main concern about this between-strain heterogeneity in the extent of live culture is that it could cause differences in sex ratio traits. There are several types of evidence indicating that this is not the case (see the DISCUSSION).

The first sex ratio was measured on all strains. We refer to the associated brood as the first brood. The experimental protocols used in the two laboratories differed slightly. In Chicago, females were simultaneously prefed on hosts and mated in groups for 24 hr. Each female was isolated and given a single host for 24 hr. In Lake Charles, females were mated in groups for 12 hr. Each female was isolated and given a single host for 24 hr, and then given two hosts on each of days two and three (except for some females of one strain that received three hosts on days two and three). The first sex ratio recorded on days two and three was that of

the composite brood. The measurement of individual females on three days allowed us to determine the between-female, within-strain component of first sex ratio variability. In both laboratories, there was approximately one male per two or three females in the mating groups. All females were between 24 and 48 hr posteclosion at the time of presentation of the first experimental host.

Second sex ratios were measured on nine strains using sequential oviposition experiments (*cf.* HOLMES 1972; WERREN 1980; ORZACK and PARKER 1986). The basic experimental unit was a single host offered for 24 hr to a female homozygous for the *stDR* eye color allele. After removal from this female, the host was offered to a wild-type female for 24 hr. Offspring of the mutant and wild-type females are distinguishable by eye color. Each wild-type female was allowed to parasitize a total of at least two hosts (offered one at a time). Females of some strains received a total of three hosts and those of one strain received four. This replication allowed us to measure the between-female, within-strain component of second sex ratio variability. For each replication, the host presented to a female was previously offered to a random *stDR* female. We note an additional point of protocol: a wild-type female remained with her vial through all of the replicates. This lessens our handling of females and prevents the second female from receiving olfactory clues from the first female other than those associated with the host.

In preparation for the sequential oviposition experiments, females of both types were mated to males of their own strain for 24 hr (in groups with a sex ratio of approximately one male per two or three females). After mating, wild-type females were each given a single host for 24 hr (August 1987) or maintained in groups for six days with fresh hosts supplied every other day (January 1988). Females were at most 48 hr posteclosion at the time of presentation of the first host. Prefeeding is a change in procedure from previous experiments (ORZACK and PARKER 1986). We regard it as somewhat artificial but pilot experiments indicated that these wildtype females would not readily parasitize previously parasitized hosts without prefeeding.

In addition to the *stDR* first sex ratios and the wildtype second sex ratios, additional sex ratios of interest in the sequential oviposition experiments are those produced by *stDR* females in hosts subsequently offered to but *not* parasitized by a wild-type female. These are first sex ratios but we call them "first-only sex ratios" in order to distinguish them from the *stDR* first sex ratios in double parasitized hosts. *stDR* first-only sex ratios are *associated* with particular wild-type strains by definition. An essential control on host characteristics is a comparison of the wildtype strains' associated *stDR* first-only sex ratios. Statistical homogeneity of such sex ratios allows one to interpret further comparisons. Any differences among the *stDR* first sex ratios in doubly parasitized hosts then reflects either some strain-specific selectivity on the part of second females with respect to hosts and/or some strain-specific effect of the second brood on the first brood.

For all experiments, the broods of a particular female were included in our analyses only if she produced a female in one of her broods. This control allows us to distinguish the all-male sex ratios of fertilized from unfertilized females.

For all analyses we assumed that adult sex ratios were the same as primary sex ratios. There is no indication of differential mortality during development in this species although additional data are needed.

We attempted to score all offspring in a brood [including the diapause larvae; see SCHNEIDERMAN and HORWITZ (1958) for methods of breaking diapause]. It is rare that less

than 95% of identifiable last instar larvae can eventually be sexed. All experiments proceeded under 24 hr light at 25°. Hosts were pupae of *Sarcophaga bullata* that were a week old or less and of a standard size (≈ 10 mm long and ≈ 3 mm wide).

Only nonparametric statistical analyses were used. Sex ratios were calculated as the proportion of females among all offspring in the brood except, of course, where we discuss diapause and direct-developing sex ratios. In several instances we present the arc-sine transformation of this proportion (SNEDECOR and COCHRAN 1980) to allow comparison with previous results. All data are available upon request.

RESULTS

First sex ratios: Our analysis was shaped by the observation that the first sex ratios produced on different days by the same female can be correlated. For the Lake Charles data, we calculated 34 out of the 36 possible Spearman rank correlations between the sex ratios produced on days one, two, and three. (*MS58* produced only a few sex ratios on day one and this day was omitted for this strain.) When $\alpha = 0.05$, 18 correlations were significantly positive and one significantly negative. All but one of the significant correlations involve sex ratios produced on consecutive days. These correlations indicate that a one-way analysis of variance on the lumped data would be inappropriate. Consequently, we analyzed the data from each day separately using one-way analysis of variance (the Kruskal-Wallis test) to detect differences among strains in sex ratios. The separate analysis of days has an additional virtue. It almost completely eliminates one source of heterogeneity: the number of hosts offered. All day one sex ratios were measured on one host while almost all day two and day three sex ratios were measured on two hosts. The only exceptions are day two and day three sex ratios produced by *MS67* females on three hosts. Kolmogorov-Smirnov tests indicate there were no significant differences ($\alpha = 0.05$) between two-host and three-host sex ratios on either day.

Given a significant Kruskal-Wallis test statistic, we carried out a two-step multiple comparison procedure (CONOVER 1980) analogous to Fisher's least significant difference procedure for Gaussian data. It controls the family-wise error rate of the comparisons at the given significance level. The results are shown in Figure 1. There is significant between-strain heterogeneity of sex ratios on each day. There is no indication of an effect of days on the strain rankings (Friedman's test, $\chi^2 = 0.744$, 2 d.f., $P = 0.689$). The increase in the proportion of significant pairwise contrasts from day 1 to days 2 and 3 suggests that between-strain differences in sex ratio are best measured over several days.

The overall average sex ratios (with 95% confidence intervals) are presented in Figure 2 along with the averages for each day. There is a significant tendency

for the day three average sex ratio to be highest (tested against the null hypothesis that any day could be highest, $\chi^2 = 8.00$, 2 d.f., $P < 0.025$). This indicates that a relative lack of sperm does not affect sex ratios over this span of time. Is there an absolute lack of sperm? Consider, for example, only those day 2 and day 3 sex ratios measured on two hosts (Table 1). For every strain, the point estimate of the average number of females increases from day two to day three (sign test: $P < 0.01$). Seven of twelve point estimates of the absolute number of males increase from day 2 to day 3 (sign test: $P > 0.05$). Hence, absolute sperm depletion does not affect first sex ratios over this span of time.

We tested for between-female heterogeneity of sex ratios within strains by applying Friedman's test to the Lake Charles data. Only those females who produced broods on all three days were included. The data form a randomized complete block design in which one can regard days one, two, and three as blocks and the individual females as treatments. Seven strains have significant between-female heterogeneity. Given a significant test statistic, we carried out a two-step multiple comparison procedure (CONOVER 1980) that controls the family-wise error rate of the comparisons at the given significance level. The results are shown in Figure 3. For the strains with significant heterogeneity, the scaled sum of ranks and average sex ratio are presented for each female along with the critical differences that separate two female's summed ranks when $\alpha = 0.05$ and $\alpha = 0.01$. The data are presented in ascending fashion to facilitate comparison of pairs of sums with the critical differences. This presentation allows one to partially answer the question: what accounts for the significant heterogeneity within strains? Comparison of the results across strains indicates that heterogeneity can be associated with differences between females that reflect the heterogeneity observed between strains. So, for example, almost all of the average sex ratios within *MS23* and *MS71* fall between 50.0 and 65.0 as do the strain averages (*cf.* Figure 2). In contrast, many of the average sex ratios within *MS67* fall outside of this range and do not reflect the between-strain heterogeneity. The reasons for the distinction between strains in their patterns of heterogeneity are not obvious. We have data suggesting that total sperm load of females is correlated with day one first sex ratio in a laboratory strain (E. D. PARKER, M. NIKLASSON and B. MONROE, in preparation). Thus, we regard significant between-female heterogeneity to be primarily a function of heterogeneity in sperm load and/or usage. We do not doubt, however, that there is segregation of alleles within these isofemale strains. It is not clear why all strains do not exhibit between-female heterogeneity. Small sample sizes might account for two nonsignificant test statistics

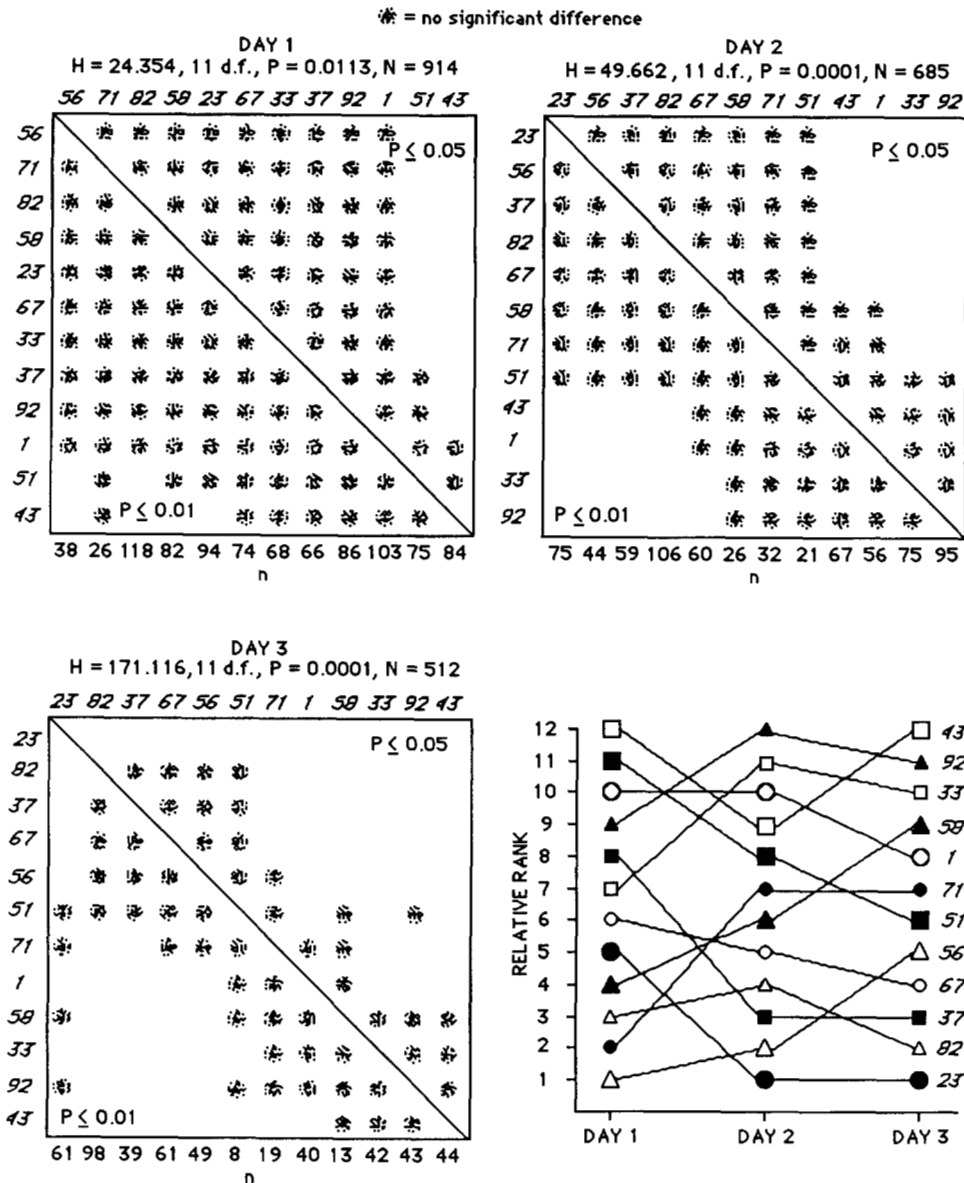


FIGURE 1.—Results of a multiple-comparison procedure applied to the first sex ratio data. The procedure is based upon a significant Kruskal-Wallis test statistic (H). For each day, we show H , the associated probability, the total sample size (N), and the sample size for each strain (n). Strains are arranged from lowest (e.g., *MS56* on day 1) to highest (e.g., *MS43* on day 1) average rank. We also plot the relative average rank for each strain on each day.

(*MS51* and *MS58*) although *MS71* has a small sample size and exhibits significant heterogeneity.

Second sex ratios: Our analysis was shaped by the observation that second sex ratios produced on different days by the same female were uncorrelated. We calculated the Spearman rank correlations between day one and day two second sex ratios. In addition, we calculated all of the possible rank correlations resulting from the day three and day four second sex ratios recorded for some strains. None of the twenty correlations were significant when $\alpha = 0.05$.

We take the lack of significant correlation of second sex ratios measured on different days to reflect their statistical independence and have lumped the data for each strain. We used the Kruskal-Wallis test to detect differences between strains in sex ratios. There is significant heterogeneity of sex ratios among the strains. Given this significant test statistic, we carried

out the two-step multiple comparison procedure as for first sex ratios. These results are shown in Figure 4. In a crude sense there is less heterogeneity than for first sex ratios since all significant differences involve two strains (*MS23* and *MS33*). As for first sex ratios, the plot of relative ranks indicates that ranks are similar across days. The overall average sex ratios are presented in Figure 5 along with the averages for each day. There is no significant tendency for the day two average to be lower than the day one average (tested against the null hypothesis that either day could be highest, $\chi^2 = 1.00$, 1 d.f., $P > 0.05$). This indicates that a relative lack of sperm does not affect sex ratios measured over this span of time. As shown in Table 2, the point estimate of the average of the absolute number of females declines in eight out of nine strains from day 1 to day 2 (sign test: $P < 0.02$). It increases from day 2 to day 3 in two of four measured strains

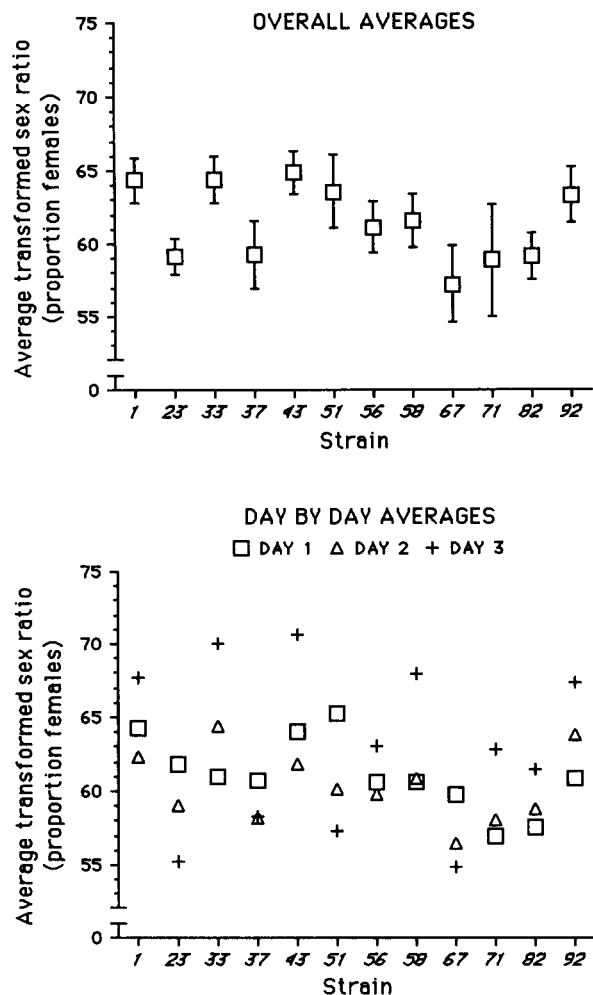


FIGURE 2.—Overall and day-by-day averages of first sex ratios. Each point represents the average value of the standard arc-sine transformation of proportion females in the brood. 95% confidence intervals are presented for the overall averages.

(not shown). In all strains these changes are paralleled by declines in the point estimates of the average of the absolute numbers of males from day 1 to day 2 (sign test: $P < 0.01$) and increases in all measured strains from day 2 to day 3 (not shown). We conclude, as in ORZACK and PARKER (1986), that sperm depletion does not affect second sex ratio (although it could affect brood size).

We tested for heterogeneity of second sex ratios within strains using Friedman's test. There are no strains with significant heterogeneity when $\alpha = 0.05$ (Table 3) in contrast to the case of first sex ratios. The reasons for this distinction are not clear. It may be due to the smaller sample sizes involved as compared to the first sex ratios although, as shown in Table 3, an "overall" test reveals no significant between-female heterogeneity. The distinction between the traits in regard to between-female heterogeneity is analyzed in the DISCUSSION.

The relationship between first and second sex ratios is of interest. As mentioned above, a weak test of local mate competition theory is to determine whether

TABLE 1

Absolute number of individuals associated with day 2 and day 3 first sex ratios (all from two hosts)

Strain	Day 2			Day 3		
	♀ mean	♂ mean	n	♀ mean	♂ mean	n
MS1	42.05	10.63	38	57.72	9.45	40
MS23	72.05	27.43	60	75.29	37.72	61
MS33	68.78	13.49	41	85.00	11.40	42
MS37	41.11	14.62	37	48.13	13.03	39
MS43	70.20	10.93	44	89.86	10.50	44
MS51	45.56	15.89	9	46.75	23.25	8
MS56	53.32	18.93	44	58.86	16.48	44
MS58	46.00	10.56	9	54.23	13.32	13
MS67	53.24	26.79	29	67.63	35.37	30
MS71	53.61	16.78	18	72.53	20.63	19
MS82	46.32	14.70	91	55.24	15.96	98
MS92	59.32	9.58	53	71.60	14.81	43

average first and second sex ratios are distinct. The data from the present study are plotted in Figure 6 along with data on other strains from ORZACK and PARKER (1986). The correlation between average first and average second sex ratios is not significant ($r_s = 0.495$, $n = 13$, $P > 0.05$). It is clear that second sex ratios are generally lower than first sex ratios. All strains produce significantly distinct distributions of first and second sex ratios (Kolmogorov-Smirnov tests, $P < 0.01$ or less, results not shown). We believe that these within-strain differences are the result of natural selection. However, we think there is little more one can infer from observing the general inequality of first and second sex ratios. Furthermore, as discussed in ORZACK (1990a, b), the existence of genetic variation for these traits makes it difficult to make "qualitative" statements about the validity of local mate competition theory.

Direct-developing vs. diapause sex ratios: Diapause larvae occurred more frequently in the present experiments than in previous experiments (PARKER and ORZACK 1985; ORZACK and PARKER 1986) in which they were never frequent enough to merit a separate analysis. The reasons for this distinction are not clear. Consequently, we regard our results as suggestive only. Experiments designed to examine the interaction between sex ratio traits and diapause tendency are clearly needed.

We used only the Chicago data in our analysis of diapause tendency. Hence, all of the data were collected under identical conditions (single hosts presented for 24 hr). In our analysis we made no distinction between broods which were entirely direct-developing or diapause and those which were mixed. Consequently, a mixed brood was scored as having separate direct-developing and diapause sex ratios.

We used the Kruskal-Wallis test to detect differences among strains in direct-developing sex ratios

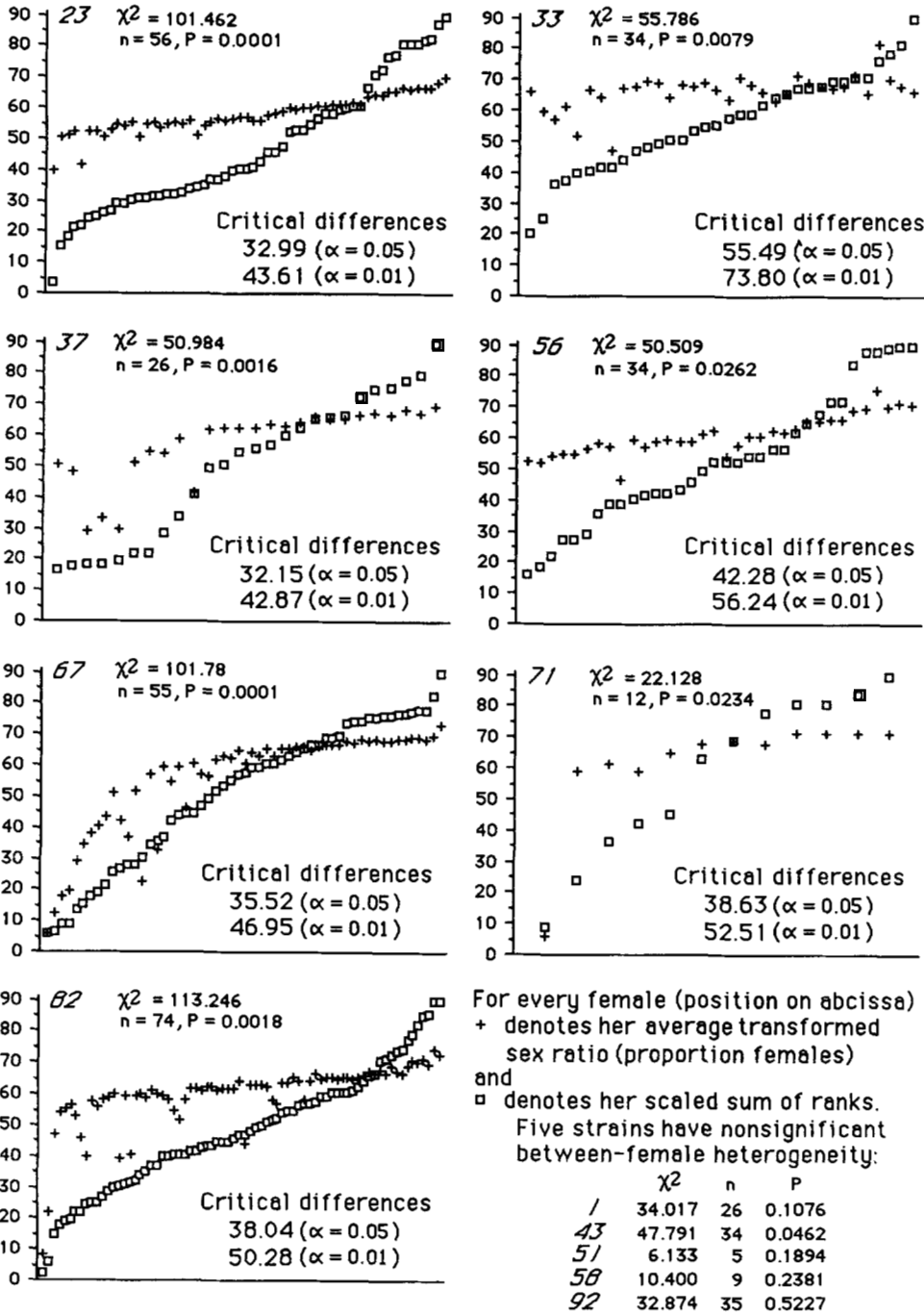


FIGURE 3.—Analysis of between-female heterogeneity of first sex ratios within strains. For all strains, we show the results of Friedman's test (χ^2), the associated probability, and the sample size (n). For those strains with a significant test statistic, we plot the average transformed first sex ratio along with the arbitrarily scaled sum of ranks of sex ratios for each female. The two points for each female are plotted one above the other. The points are arranged in ascending order to facilitate comparison with the critical differences that separate significant sums of ranks at the given α level.

and in diapause sex ratios. There was significant between-strain heterogeneity for all combinations of sex ratios and developmental types (Table 4).

As shown in Figure 7 (top), average diapause and direct-developing sex ratios are similar regardless of whether they are first or second sex ratios. The correlation between the direct-developing and diapause sex ratios is significant for the first sex ratio ($r_s = 0.648$, 95% confidence interval: 0.009–0.911) but not for the second sex ratio ($r_s = 0.150$, 95% confidence interval: -0.587–0.751).

One can also see in Figure 7 (bottom) that average first and second sex ratios are similar regardless of

developmental type. The correlations between the two sex ratios are not significant or distinct (diapause $r_s = 0.143$, direct-developing $r_s = 0.467$, $\chi^2 = 0.338$, 1 d.f., $P > 0.05$). In addition, the results of Kolmogorov-Smirnov tests indicate that there were significant differences between direct-developing and diapause distributions for some strains for both first and second sex ratios (Table 5).

Additional insight concerning developmental type and sex ratio can be gained by the analysis of mixed broods. The correlations between direct-developing and diapause sex ratios of such broods are shown in Table 6. The χ^2 values listed for each strain (each

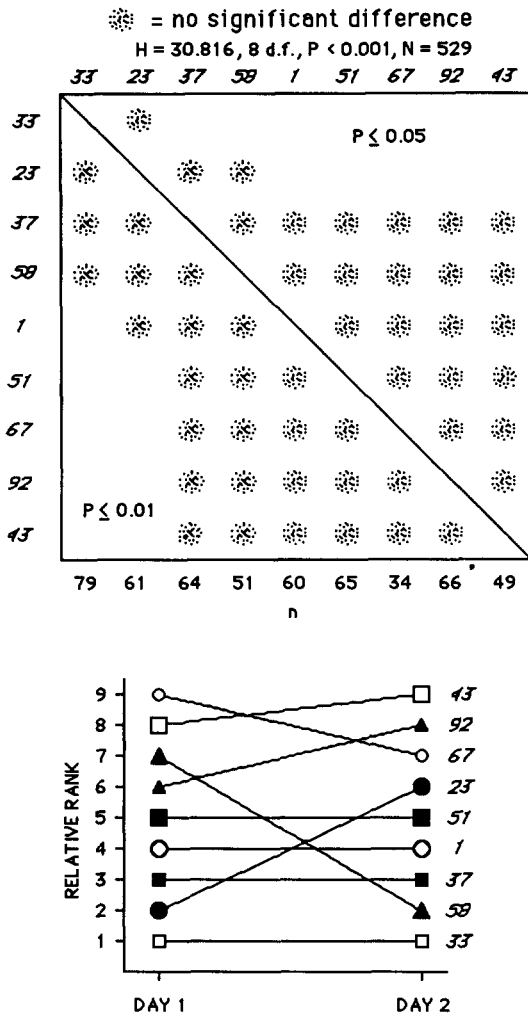


FIGURE 4.—Results of a multiple-comparison procedure applied to the overall second sex ratios. The procedure is based upon a significant Kruskal-Wallis test statistic (H). We show H , the associated probability, the total sample size (N), and the sample size for each strain (n). Strains are arranged from lowest ($MS33$) to highest ($MS43$) average rank. We also plot the relative average rank for each strain for days 1 and 2.

with 1 d.f.) indicate that there were no significant distinctions between the within-female correlations for first and second sex ratios when $\alpha = 0.05$. The common within-female correlations for each sex ratio were also not significantly distinct when $\alpha = 0.05$.

stDR first-only broods vs. stDR first broods: We used the Kruskal-Wallis test to compare the *stDR* first-only sex ratios as well as the *stDR* first sex ratios associated with each strain. The former are not significantly different ($H = 14.899, 8 \text{ d.f.}, P > 0.05, N = 248$). This result indicates that the between-strain differences in second sex ratios reflect differences manifested in response to the same experimental conditions. There is significant heterogeneity of the *stDR* first sex ratios ($H = 20.425, 8 \text{ d.f.}, P < 0.01, N = 529$). Given the homogeneity of *stDR* first-only sex ratios, we believe this heterogeneity reflects the strain-specific way in which a second female affects the brood

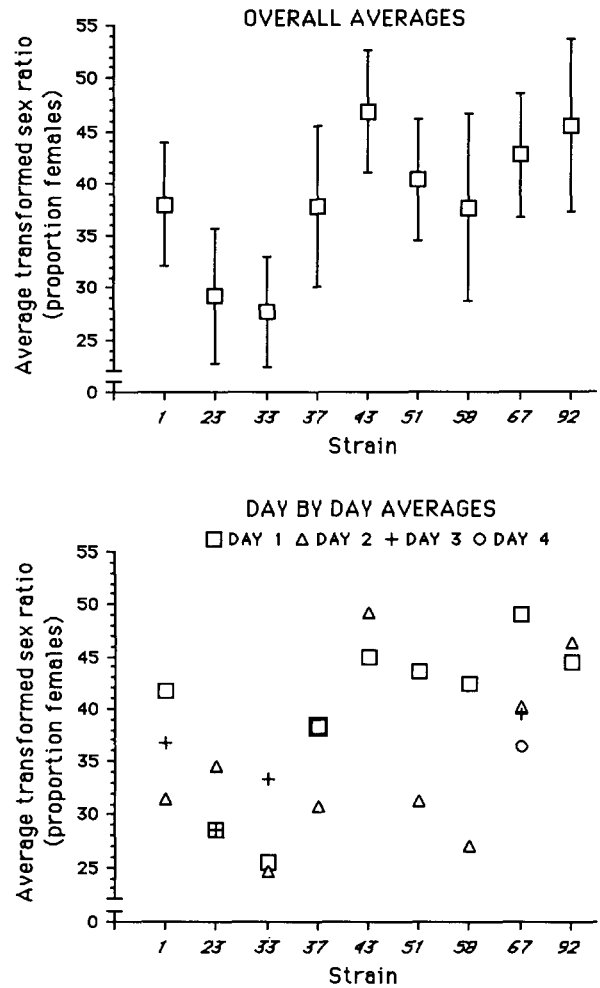


FIGURE 5.—Overall and day by day averages of second sex ratios. Each point represents the average value of the standard arc-sine transformation of proportion females in the brood. 95% confidence intervals are presented for the overall averages.

of the first female. The multiple comparison procedure indicates that nine of 36 comparisons are significant when $\alpha = 0.05$ and five of 36 when $\alpha = 0.01$. Six of the former comparisons and four of the latter involve $MS33$.

Similar heterogeneity occurs among the *stDR* first brood sizes ($H = 33.888, 8 \text{ d.f.}, P < 0.001, N = 529$) although there is significant heterogeneity among the *stDR* first-only brood sizes ($H = 4.773, 8 \text{ d.f.}, P > 0.05, N = 248$). The multiple comparison procedure indicates that 13 of 36 comparisons are significant when $\alpha = 0.05$ and eight of 36 when $\alpha = 0.01$. Curiously the strains associated with distinctive *stDR* first sex ratios are generally not those associated with distinctive *stDR* first brood sizes. $MS1$ or $MS67$ is involved in ten of the 13 significant comparisons when $\alpha = 0.05$ and seven of the eight significant comparisons when $\alpha = 0.01$.

These effects and their differences among strains are intriguing but they lack a clearcut evolutionary interpretation (see below).

TABLE 2

Absolute number of individuals associated with day 1 and day 2 second sex ratios

Strain	Day 1		<i>n</i>	Day 2		<i>n</i>
	♀ mean	♂ mean		♀ mean	♂ mean	
<i>MS1</i>	8.54	7.36	39	3.89	4.05	19
<i>MS23</i>	7.77	12.51	35	4.57	3.93	14
<i>MS33</i>	2.90	7.00	21	3.18	6.18	22
<i>MS37</i>	7.78	8.58	36	2.67	5.53	15
<i>MS43</i>	10.50	9.56	36	6.29	4.33	24
<i>MS51</i>	9.32	8.57	47	4.33	4.11	18
<i>MS58</i>	15.78	9.78	23	2.00	6.64	11
<i>MS67</i>	13.43	6.87	23	6.54	5.61	13
<i>MS92</i>	7.43	7.40	30	5.89	5.10	19

DISCUSSION

It is difficult to compare our results with previous work given the paucity of information concerning genetic variation for sex ratio traits within natural populations. We hope our results will provide an impetus for similar studies in other organisms (particularly other parasitic Hymenoptera).

We address in turn the issues raised in the RESULTS section.

Between-strain heterogeneity in diapause history:

As outlined in MATERIALS AND METHODS the strains differed in the number of generations of live culture prior to the experiments. Do such differences cause the between-strain differences in sex ratios? We think not given our comparisons of strains with different histories. So, for example, *MS23* and *MS58* have different histories but have similar average first sex ratios (although *MS23* stands out on day three). Conversely, two strains with similar histories (*MS37* and *MS43*) have different first sex ratios. These conclusions also apply to second sex ratios: strains with different histories may have similar sex ratios (compare *MS23* and *MS33* in Figure 5) and strains with similar histories may have different sex ratios (compare *MS33* with *MS43* in Figure 5). More generally, the average behaviors (cf. Figure 6) of relatively new strains are similar to the behaviors of long-established laboratory strains (e.g., + and *stDR*). This similarity implies that the between-strain variation in sex ratio behaviors is "real."

Genetic variation for first sex ratios: There are several ambiguities in our understanding of the evolutionary consequences of this variation. The first relates to the forces that govern sex ratio evolution. Much of our previous work and that of others relies on the assumption that local mate competition (HAMILTON 1967) is the process governing sex ratio evolution in this species. This may well be true. An alternative is to believe that sibmating within populations is the process governing the evolution of sex ratios.

TABLE 3

Analysis of between-female, within-strain heterogeneity of second sex ratios

Strain	χ^2	No. of days	<i>n</i>	<i>P</i>
<i>MS1</i>	20.135	3	16	0.1668
<i>MS23</i>	8.255	3	10	0.5087
<i>MS33</i>	13.470	3	11	0.1986
<i>MS37</i>	13.534	2	13	0.3315
<i>MS43</i>	21.902	2	21	0.3459
<i>MS51</i>	13.846	2	15	0.4612
<i>MS58</i>	3.850	2	8	0.7969
<i>MS67</i>	7.878	4	6	0.1631
<i>MS92</i>	17.140	2	17	0.3766
Overall	124.504	2	117	0.2780

See UYENOYAMA and BENGTTSSON (1982, p. 62) for a comparison of these processes. Can the data help us determine their relative importance in this species? We think not given the following argument. Consider a population of haplodiploid females each with independent control of its sex ratios. With local mate competition, a genetic model (TAYLOR and BULMER 1980) indicates that the optimal sex ratio of HAMILTON (1967) is stable to invasion by a rare mutation with any other sex ratio. This implies that a true polymorphism is not possible although an interior stable equilibrium has not been ruled out. With sibmating, a genetic model (UYENOYAMA and BENGTTSSON 1982) indicates that the optimal sex ratio is unstable to invasion. This implies that a true polymorphism may be possible although this has not been proven. Unfortunately, these distinct predictions are not useful in practice for several reasons. First, it is not clear whether the first sex ratio variation we observed is stably maintained. Second, either or both theoretical results may not apply to the case of multiple alleles. Third, we lack information on the genetic nature of the between-strain differences.

Further evolutionary insight can be gained by determining the fitness consequences of the first sex ratio variation. This exercise is essential because it indicates the range of fitness variation that must be reconciled with present theory or indicates what discrepancies between theory and data are *not* to be explained. We regard the latter assessment to be particularly important in judging the utility of optimality models of sex ratio evolution.

In this regard, we believe it is not meaningful to state that a particular sex ratio provides qualitative support for local mate competition theory by being within, say, ten or twenty percent of the optimum or equilibrium value. Such a statement sidesteps the critical issue of what is the expected nature of fit of the data to the predictions (ORZACK 1990a, b).

Aside from this important issue of interpretation, there remain ambiguities in this type of fitness "cali-

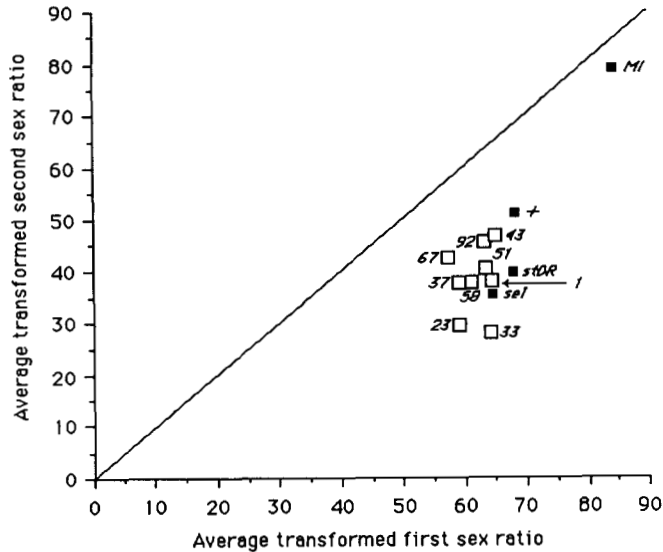


FIGURE 6.—The relationship between average transformed first sex ratio and average transformed second sex ratio. We also plot points for four strains measured previously (ORZACK and PARKER 1986).

bration." In particular, calculation of fitnesses of first sex ratios in populations with local mate competition or sibmating requires assumptions about the inbreeding coefficient (F), the distribution of foundress numbers, and the associations of foundresses within groups. At present, there is little information about these quantities or distributions in this species. Consequently, we regard the assumptions underlying the following calculations as just one of several sets of plausible assumptions. Note especially that we analyze first sex ratio behavior by itself. This is a pragmatic decision given that the relative contributions of first and second sex ratio behaviors to total fitness are unknown.

We assume that no mixed groups of foundresses occur. In particular, each female (assumed to be true-breeding) is the sole contributor of offspring to a mating pool. Given this assumption, the absolute fitness of a particular female is then either directly equal to ($F = 0$) or proportional to ($F \neq 0$) the sex ratio itself [see, for example, FRANK (1986) Equation 5]. Accordingly, differences between strains with respect to sex ratios translate directly into differences between strains with respect to fitness. For example, the significant heterogeneity observed between strains with respect to first sex ratio distributions (Figure 1) indicates that the strains are heterogenous with respect to fitness. Additionally, the differences in overall average first sex ratio (Figure 2) indicate that the average fitnesses of the strains differ.

What do these differences imply for our understanding of sex ratio evolution? This is a more difficult question to answer which relates to another ambiguity: the nature of the predictions of optimality and genetic models of local mate competition. Their joint

TABLE 4

Analysis of between-strain heterogeneity of sex ratios associated with developmental type

Sex ratio	Developmental type	H	d.f.	P	N
First	Direct-developing	26.092	10 ^a	0.0036	601
	Diapause	21.861	9 ^{a,b}	0.0106	196
Second	Direct-developing	27.591	8	0.0006	236
	Diapause	21.674	8	0.0056	406

^a MS56 was not analyzed in this experiment.

^b MS43 did not produce any diapause larvae.

prediction for the situation outlined above is that the brood should be entirely female. This prediction is usually interpreted as implying that a female produce the minimum number of males necessary to inseminate the females in the mating group. This may be a reasonable qualitative interpretation of the theory but it raises some difficult questions. What, for example, accounts for the diversity of average fitnesses associated with the range of average first sex ratios shown in Figure 6? To put it another way, why aren't all strains like *MS41*? Another important question is: how much fitness variation is allowable under this qualitative interpretation of the theory? There are two ways to calculate relative fitnesses for these strains. The first is in a strong sense: relative to the optimum (100% female). This is justified by the observation that very female-biased first sex ratios can evolve (e.g., *MS41*). The absolute fitness of the optimum sex ratio is 1.0. Accordingly, *MS43*, for example, has a relative average fitness of 0.654. Alternatively, one can calculate the relative average fitnesses in a weak but more realistic sense by dividing the absolute fitnesses by the highest fitness among them. Then, for example, *MS67* has the lowest relative fitness ($0.876 = 57.242/65.369$) while *MS43* has the highest ($1.000 = 65.369/65.369$). Both calculations imply that the population is not at selective equilibrium. In our opinion, this range of relative fitnesses should at least give pause to a biologist who regards a difference in relative fitnesses of 1% or so to be evolutionarily important.

Our overall perspective concerning these results is simple. We believe they demonstrate the need for better information on sex ratio variation in this (and other) species, the need for further study of the dynamics of models of sex ratio evolution, and especially the need for debate about the manner in which present models should be used and their success judged. In regard to the last point, it is clear that our present theory of sex ratio evolution provides useful but limited insights into our data. At the same time, however, we have gained insights and raised important questions by learning what theory does not account for (i.e., fitness variation among strains). We regard such knowledge of failure as essential for judging the extent

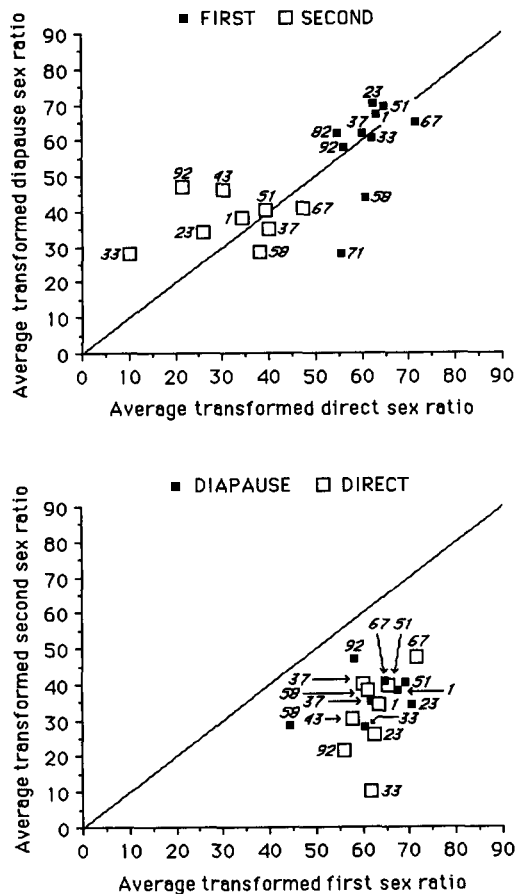


FIGURE 7.—Relationship between sex ratios and diapause tendency. At top, we present average transformed direct-developing sex ratio versus average transformed diapause sex ratio for first and second sex ratios. At bottom, we present average transformed first sex ratio vs. average transformed second sex ratio for both developmental types.

to which present theory can be deemed successful (ORZACK 1990b).

The association between first sex ratios and second sex ratios: As shown in Figure 6 these traits are uncorrelated between strains. One of our goals is to analyze strains from other populations to determine whether this pattern is generally true. We have no information about within-female correlations between first and second sex ratios. Such data are a prerequisite to answering several questions: what are the relative contributions of first and second sex ratio behaviors to total fitness? Does natural selection for one enhance or compromise the other? Does the general independence observed between strains reflect the action of selection? Do between-strain and within-female patterns of correlation differ? Are the former heterogeneous between populations? Are the latter heterogeneous within populations? If so, what do these patterns reveal about evolutionary dynamics?

Between-female heterogeneity of sex ratios: We detected significant between-female heterogeneity for first sex ratios (Figure 3) but not for second sex ratios

TABLE 5

Analysis of heterogeneity of sex ratio distributions between developmental types within strains

Sex ratio	Strain	Direct-developing <i>n</i>	Diapause <i>n</i>	Maximum difference	<i>P</i>
First	MS1	93	8	0.750	<0.05
	MS23	52	8	0.462	>0.05
	MS33	47	27	0.147	>0.05
	MS37	37	29	0.171	>0.05
	MS51	73	38	0.393	<0.05
	MS58	94	39	0.464	<0.05
	MS67	3	15	0.800	>0.05
	MS71	26	8	0.500	>0.05
	MS82	52	6	0.481	>0.05
	MS92	53	17	0.109	>0.05
Second	MS1	19	71	0.157	>0.05
	MS23	53	17	0.235	>0.05
	MS33	15	63	0.448	<0.05
	MS37	22	41	0.280	>0.05
	MS43	28	52	0.387	<0.05
	MS51	31	43	0.188	>0.05
	MS58	19	21	0.308	>0.05
	MS67	30	53	0.154	>0.05
	MS92	19	45	0.462	<0.01

(Table 3). A possible explanation for this distinction is that a second female faces uncorrelated between-host differences in first brood size and sex ratio to which she responds in an uncorrelated manner. This is plausible because almost surely a different *stDR* female parasitized each of the hosts parasitized by a particular second female. In contrast, a first female faces a uniform environment. One assumption underlying this argument is that a second female can perceive these extrinsic differences. The second is that the effects of such differences on female behavior are greater in magnitude than those of the uncorrelated differences in intrinsic host characteristics. One way to test this explanation is to offer a second female hosts parasitized by the same *stDR* female. Presumably, this results in a more uniform environment for the second female and between-female heterogeneity of second sex ratios might be detectable.

We note that the ability of second females to produce a precise second sex ratio is poor although there are differences between strains in this regard (ORZACK 1986, 1990a). Hence, we do not mean to imply that the lack of between-female heterogeneity for second sex ratios indicates precise "host by host" sex ratio adjustment. Indeed, we believe that this lack of canalization could be due to a history of weak and/or intermittent natural selection for this trait.

Direct-developing versus diapause sex ratios: We start by asking: what kind of association between these traits is expected? Consider the first sex ratio. Ideally, a female would have the ability to adjust the sex ratios of both components of her brood to reflect the mating conditions prevailing upon their separate emergences.

TABLE 6
Analysis of within-female correlations between direct-developing and diapause sex ratios

Strain	Sex ratio						χ^2
	First ^a			Second ^b			
	r_s	95% C.I.	n	r_s	95% C.I.	n	
MS1	0.760	0.093–0.956	8	0.694	0.141–0.917	11	0.574
MS23	-0.128	-0.774–0.649	8	0.553	-0.198–0.895	9	1.453
MS33	0.321	-0.186–0.693	18	0.145	-0.432–0.638	14	0.209
MS37	0.872	-0.085–0.992	5	0.530	-0.082–0.852	12	0.871
MS43 ^c				0.286	-0.193–0.655	20	
MS51	0.151	-0.225–0.488	31	0.026	-0.663–0.691	9	0.000
MS58	0.380	0.043–0.639	35	-0.223	-0.883–0.734	6	1.017
MS67 ^c				0.418	-0.094–0.755	17	
MS71	-0.338	-0.908–0.671	6				
MS82	0.316	-0.934–0.982	4				
MS92	0.029	-0.813–0.832	6	0.147	-0.409–0.623	15	0.032
Common	0.294	0.094–0.470		0.335	0.130–0.513		0.981
Overall common	0.314	0.172–0.442					

^a $\chi^2 = 7.505$, 8 d.f., $P > 0.05$. C.I. = confidence interval.

^b $\chi^2 = 5.439$, 8 d.f., $P > 0.05$.

^c These strains did not produce any mixed first broods.

The only direct information she could have about the future mating group is derived from information she has about the number of foundresses she encounters and about their broods. Hence, the same first sex ratio might be appropriate for the direct-developing and diapause broods. This implies a strong association between the two. In contrast, we observed weak correlations between direct-developing and diapause sex ratios within females (Table 6) and between the average direct-developing and diapause first sex ratios (Figure 7, top). The problem with the prediction is its assumption that all females contribute equally to both broods. There are differences between these strains with respect to diapause tendency (E. D. PARKER and M. NIKLASSON, unpublished). Hence, it may be inappropriate for a female to "assume" that other females will contribute to the future mating group in an equal manner. So, the weak association between direct-developing and diapause sex ratios might be an evolutionary response to the expected weak association between present ecological conditions and those in the future.

The decision faced by second females about direct-developing and diapause sex ratios is similar to that faced by a first female. Presumably, any sex ratio appropriate for the direct-developing component of the brood might be appropriate for the diapause component. This assumes again that diapause only causes a temporal delay in the occurrence of local mate competition. Alternatively, a second female might "uncouple" the two sex ratios in anticipation of a change in the degree of local mate competition expected upon emergence. At the extreme she might produce a diapause second brood with a first-sex-ratio-

like sex ratio when confronting a completely direct-developing first brood.

None of this speculation is compelling. Further information concerning the variation in diapause tendency among females contributing offspring to local mating groups is obviously needed.

stDR first-only broods vs. stDR first broods: Our assumption is that the homogeneity of *stDR* first-only sex ratios and brood sizes reflects the uniformity of hosts. Consequently, the heterogeneity of *stDR* first sex ratios and brood sizes reflects differences between the wildtype strains in their effects on first broods.

There are at least two naive evolutionary expectations as to the nature of such effects. A second female could reduce the number of sons of the first female in order to increase the share of inseminations to be had by her sons in the local mating group. Another possibility is that a female reduce the numbers of male and female offspring of the first female in order to increase the brood size ratio. How do these naive expectations accord with the data? Six of nine point estimates of average sex ratios for first broods are larger relative to those for first-only broods (sign test: $P > 0.05$) implying that the former contain fewer males. Seven of nine point estimates of average first brood sizes are smaller relative to those for first-only broods (sign test: $P = 0.05$). Hence, there is a weak indication that second broods affect first brood sizes more than they affect first sex ratios.

A crude idea of the relative benefits of these different effects of second broods could be gained by examining the fitness of a second sex ratio as a function of the first brood size and sex ratio (*cf.* WERREN 1980). However, the changes in fitness resulting from alter-

ation of the first sex ratio or first brood size also depend upon the second brood size and sex ratio. Hence, it is difficult to make general statements about which change is more beneficial.

We finish with a caution about a danger of this sort of explanation for the interaction between broods. One can say that it is beneficial for a second female to manipulate a first female's brood. This may indeed be an appropriate explanation of *MS33*'s behavior. Yet, such an explanation would not be complete until it is clear why other strains do not interact in this manner.

The general significance of our results: Clearly we have an incomplete understanding of sex ratio evolution in this species. Many unanswered questions concerning data and theory have been outlined above. Our intent is not to tout our ignorance but rather to give pause to those who want to simply characterize the behaviors of this species as optimal (*e.g.*, SUZUKI and IWASA 1980; WERREN 1980, 1983; CHARNOV 1982; THORNHILL and ALCOCK 1983; see also WERREN 1987). We are not motivated by a belief that theories of sex ratio evolution based upon optimality principles are unimportant. Instead, our motivation is the belief that progress in the study of sex ratio evolution will be made only by knowing the limits of our understanding of empirical patterns.

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