Quantitative Genetics of Sex Ratio Traits in the Parasitic Wasp, *Nasonia vitripennis*

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Manuscript received May 30, 1993
Accepted for publication January 20, 1994

ABSTRACT

We detected significant parent-offspring regressions for the first sex ratio (the sex ratio produced by a female in a fresh host) and the second sex ratio (the sex ratio produced by a female in a previously parasitized host) in the parasitic wasp, *Nasonia vitripennis*. For both traits, estimates of the narrow-sense heritability range from ≈0.05 to ≈0.15 (depending on how the data are analyzed). The study population was derived from isofemale strains created from wasps captured in a single bird nest. The same population exhibited no significant parent-offspring regression for the brood sizes associated with the first and second sex ratios. There may be a significant negative parent-offspring regression for diapause proportion in the first sex ratio broods. The estimates of the genetic correlations between first and second sex ratios are positive although almost all are not significantly different from 0.0. To our knowledge, this study is the first "fine-scale" analysis of genetic variation for sex ratio traits in any species of insect. Such studies are an essential part of the assessment of the validity of claims that sex ratio traits are locally optimal.

DESPITE considerable progress in our empirical and theoretical understanding of sex ratio evolution (see WRENSCH and EBBERT 1992), there are considerable gaps in our knowledge. For example, we know little about the nature and extent of genetic variation for sex ratio traits. This is especially true for species having a clear potential for such variation because their sex ratios are known to be highly variable and to be strongly affected by environmental conditions. Examples of such species include those Reptilia with environmental sex determination (see JANZEN and PAUKSTIS 1991) and those Hymenoptera that are haplodiploid.

Previous analyses of genetic variation for sex ratio traits in species of Hymenoptera are limited in important ways. Some studies have assessed genetic variation at the species level by analyzing laboratory populations generated by the amalgamation of geographically distinct strains (WILKES 1947; SIMMONDS 1947; DAS 1959; SASTRY 1962; RAM and SHARMA 1977; PARKER and ORZACK 1985; ANTOLIN 1992a,b). Other studies have looked for genetic variation at the species or populational level by comparing isofemale strains (ORZACK and PARKER 1986, 1990; ORZACK *et al.* 1991). No studies have determined the narrow or broad sense heritability of sex ratio traits *within* what may be a natural population. This was the purpose of the present experiment in which we measured two traits: the first sex ratio (the sex ratio produced by a female in a fresh host) and the second sex ratio (the sex ratio produced by a female in a previously parasitized host).

This kind of information on the heritability of sex ratio traits within local populations is of interest in several evolutionary contexts. First, it is of interest to know whether the haplodiploidy of most Hymenoptera has the consequence that populations of such species have less genetic variation than do the populations of otherwise generally similar diplodiploid species. Such information has an obvious relevance to the debate over the adaptive nature (if any) of genetic variation within natural populations since the haploidy of males results in the potential for more selective scrutiny of rare alleles than if they were almost always present only in heterozygous form, as is expected if a species is diplodiploid. Second, there are many claims that sex ratio traits in this and other species are locally optimal. Assessing the heritability of these traits within populations (the evolutionary domain in which local optimality is defined) is an essential component of assessing the validity of such claims. (Further discussion of these and related issues are provided in the DISCUSSION.)

MATERIALS AND METHODS

In June 1990, all female wasps emerging from dipteran hosts (pupae of *Apaulina* sp.) present in a *single* great tit nest were allowed to mate with emerging males and then to separately oviposit on hosts under conditions leading to the production of diapause larvae. A total of 119 isofemale strains were created in this manner. The nest originated ≈5 km. south of the locality near Södertälje, Sweden, that was the source of the isofemale strains studied in ORZACK and PARKER (1990) and ORZACK *et al.* (1991).

Construction of the base population: In April 1991, hosts were removed from storage at 4° and stored at 25° so that the diapause larvae could complete their development. When the larvae pupated and were sexable, three virgin females and two virgin males from a single host were collected for each strain. Eleven strains lacked either three females or two males and were not used subsequently. The females and males chosen from the remaining 108 strains were combined randomly into groups of 10 or 11 females and 6 or 7 males. Each of the

resulting 30 vials was given 10 fresh hosts. The hosts were removed after 3 days and replaced with an equal number of fresh hosts. This was repeated after intervals of 2, 2 and 3 days.

Measurement of sex ratios in the parental generation: Females (mothers) were chosen at random from the pupae produced in the second and fourth sets of hosts given to the females of the base population (see above) so that the experiment could be spread over 3 days (with ≈500 mothers set up on each day). A total of 1500 mothers was estimated to be an adequate sample size to detect a significant regression coefficient of ≈0.07. This target value for the coefficient comes from a previous analysis of the first sex ratio (PARKER and ORZACK 1985). The sample size estimate accounts for the correlation between the sex of individuals within and between broods and for sampling variability of the daughters' sex ratios (S. H. ORZACK and R. CHAPPELL, submitted for publication), and for the mortality and noninsemination rates of females.

For both of the two sets used, hosts from each of the 30 vials were chosen randomly and in almost all cases, 10 mothers were taken as pupae from each host, the goal being that a large number of females contribute mothers to the parental generation. Males were chosen randomly from each of ten base population vials. Within 24 hr of emergence, groups of ≈40 mothers were given ≈40 males and 5–10 fresh hosts to allow mating and prefeeding before use. Prefeeding is a possibly artificial feature of this experiment and some previous experiments (ORZACK and PARKER 1990; ORZACK *et al.* 1991) but it greatly simplifies the handling of large numbers of females. Mothers were prefed for up to 5 days before use (approximately one-third for 1 day, one-quarter for 2 days, one-third for 3 days, and one-tenth for 5 days).

After prefeeding, individual mothers were put on single hosts in order to measure their first sex ratios. On the same day, in order to create the previously parasitized hosts necessary for the measurement of second sex ratios, individual *stDR* females (previously mated to *stDR* males) were given single hosts to parasitize for 24 hr. Many of these *stDR* females were used more than once during this part of the experiment. The scarlet eye color of *stDR* individuals is distinct from that of wild-type individuals.

To measure the mother's second sex ratio, the first-sex-ratio host was removed after 24 hr and replaced with a host previously parasitized by an *stDR* female. This host was removed after 24 hr.

Measurement of sex ratios in the offspring generation: Twelve days after each set of 500 first sex ratio hosts were set up in the mother generation, four daughter pupae were isolated from each successfully parasitized host. Within 24 hr of emergence, the four daughters of each brood were given one host and four males in order that they be mated and fed prior to the measurement of first sex ratios. Males were taken randomly from the first sex ratio broods that contained no females and from mixed-sex broods in such a way that contributions from each brood were approximately equal. After 24 hr, one daughter of the four was chosen randomly and given a fresh host in order to measure her first sex ratio. Only one daughter was measured because any positive correlation between the sex of individuals making up the daughters' broods implies that the sampling variance of the regression coefficient is more efficiently reduced by increasing the number of mothers measured as opposed to increasing the number of daughters measured per mother (S. H. ORZACK and R. CHAPPELL, submitted for publication). On the same day, individual *stDR* females (previously mated to *stDR* males) were given single hosts to parasitize for 24 hr. To measure the daughter's second sex ratio, the first host was removed after 24 hr and replaced with a host previously parasitized by an *stDR* female. This host was removed after 24 hr.

An important aspect of our experimental design relates to the mating of females. When estimating the heritability of a sex ratio trait putatively dependent only on the mother, random mating should occur in the base population (to produce the mothers) and in the mother generation (to produce the daughters). In the base population, females and males chosen from each original strain were combined randomly into groups. In the mothers' generation, males from all of the 30 base population vials were combined randomly with groups of virgin females. Both of these procedures should reasonably approximate random mating for the population. Group matings of this kind were not possible in the daughter generation (because daughters must be correctly assigned to mothers). However, they are not required for a proper heritability estimate if our assumption about the maternal determination of sex ratio is correct. Nonetheless, we did randomly assign males to each vial of daughters (see above). The main reason was that although the haplodiploid mechanism of sex "determination" is often taken to imply that the male genotype has no effect on the sex ratio of his mate, there is evidence from another species that a male's genotype can affect the oviposition behavior of his mate (LEGNER 1988, 1989) and the general potential for such influences is clear [see HAWKES (1992) and ORZACK (1992) for relevant discussions].

An additional important aspect of our experimental design relates to the source of males used for mating. In the base population, all broods contributed males, while in the mother generation, all of the females present (mothers and nonmothers) potentially contributed males. In the daughter generation, most of the males used for mating were the offspring of mothers who produced no daughters. A possible complication is that these mothers may have produced no daughters because of their genotypes and not because they were unmated. If this were true, some of the genetic variability for sex ratio traits in the mother generation would have been overrepresented in the daughter generation.

In both generations, diapause larvae were present in some of the broods. In some instances, the mother's first sex ratio brood was composed entirely of diapause larvae (thereby preventing measurement of the daughter's sex ratios at the time of the original experiment). These diapause larvae were stored at 4° and allowed to complete development in June 1992. The daughter's first and second sex ratios were then measured using the protocol described above. All other diapause larvae from the original experiment were stored at 4° and allowed to complete development in November 1991. Diapause larvae of the daughters' broods measured in June 1992 (see above) were stored at 4° and allowed to complete development in October 1992.

A previous study (ORZACK and PARKER 1990) described the effects of the second (wild type) brood in the host on the first brood (*stDR*). In order to assess these effects further, control sex ratios were created by giving single hosts to individual *stDR* females for 24 hr.

All phases of the experiment proceeded at 25° and 24 hr daylight. Hosts were pupae of *Sarcophaga bullata* of standard size (≈10 mm long and ≈3 mm wide) that were 10 days old or less. (Pupae of *Calliphora vomitoria* were used to make the original set of isofemale strains in June 1990.) We used 10 dram shell vials for prefeeding and group matings and 10 × 75-mm disposable culture tubes stoppered with cotton plugs for single-female matings and sex ratio measurements. Since all-male broods might result simply from a female not being inseminated, the broods of a female were included in the data analysis only if she produced at least one female.

The second sex ratio brood of a particular female was included in our analysis only if *stDR* offspring were present in the host (not all ovipositions by *stDR* females were successful).

TABLE 1

Values of the regression coefficient, β, relating mother and daughter sex ratios

	First sex ratio				Second sex ratio			
	Direct		Total		Direct		Total	
Constant	β	χ^2	β	χ^2	β	χ^2	β	χ^2
0.01	0.077	6.15[a]	0.081	6.16[a]	0.075	7.30[b]	0.073	7.56[b]
0.001	0.057	6.18[a]	0.060	5.00[a]	0.050	6.97[b]	0.050	7.42[b]
0.0001	0.043	5.98[a]	0.044	4.03[a]	0.037	6.74[b]	0.037	7.25[b]
0.00001	0.034	5.75[a]	0.033	3.30	0.030	6.58[a]	0.029	7.18[b]
None	0.095	4.56[a]	0.059	2.16	0.092	2.51	0.101	3.39
n	396		517		214		242	

Entries alongside constants stem from logistic regression analysis. Entries alongside "none" stem from least-squares regression analysis of arc-sine transformed values. The χ^2 value measures the effect of adding β to the regression model. Each value has one degree of freedom. n denotes sample size.

[a] $P < 0.05$.

[b] $P < 0.01$.

The few second sex ratio broods that were accompanied by an all-male *stDR* brood were included.

All individuals in a brood were used in an analysis if possible. Occasional individuals were not sexable or could not be genotyped. Most such individuals were pupae that died prior to emergence or diapause larvae that failed to eclose. These individuals were included in analyses of brood size and diapause proportion. One consequence is that the sample size associated with the regression analysis of second-sex-ratio brood sizes is larger than that associated with the total second sex ratio (see below). When unscorable diapause larvae could be of two genotypes (as in second-sex-ratio hosts), they were recorded as wild type since *stDR* females very rarely produce diapause larvae.

Statistical procedures: We used least-squares regression analysis of arc-sine transformed values and logistic regression analysis to assess the relationship between mother and daughter sex ratios (proportion males) and between mother and daughter diapause tendencies (proportion diapause). Significant advantages of logistic regression analysis are (1) arc-sine transformation does not usually "normalize" distributions of sex ratios in this species, (2) more accurate responses (those daughter sex ratios or diapause proportions associated with larger brood sizes) are given more weight in the estimation procedure, and (3) one can account for overdispersion of proportions (relative to binomial variance) [see CROSBIE and HINCH (1985), MCCULLAGH and NELDER (1989), ORZACK (1990) and THOMPSON (1990) for further statistical and biological details]. In the present context, these features make logistic regression analysis superior to least-squares analysis of arc-sine transformed values.

A constant was added or subtracted to all sex ratios and diapause proportions of mothers when they were used as predictors. This ensures that the logit of each predictor is finite. The constant was added to first sex ratios since many first sex ratios are equal to 0.0. The constant was subtracted from all second sex ratios since many second sex ratios are equal to 1.0. (The constant was added to the few all-female second sex ratios.) A constant was added to all diapause proportions since many are equal to 0.0. (The constant was subtracted from the few all-diapause broods.) The value of the constant was varied over four orders of magnitude. The effects of the value of the constant on the regression analyses are shown below.

To calculate the genetic correlations between the sex ratio traits, we estimated the cross-covariances either by logistic regression (by multiplying the regression coefficient of the daughter's trait on the mother's trait times the variance of the mother's trait) or directly from the arc-sine transformed values. Given a cross-covariance, we used standard formulae for estimating the genetic correlation and its standard error (*e.g.*, see BECKER 1984). In addition, we used a bootstrap resampling technique with 1000 replications to determine the 95% confidence interval for the estimate of each genetic correlation. This interval was estimated by the percentile method and by the bias-corrected percentile method [see EFRON and TIBSHIRANI (1986) and BANKS (1989) for further details].

Least-squares regression analysis was used to assess the relationship between mother and daughter brood sizes. Other statistical analyses involved standard nonparametric tests (see below). For all tests, the α value for determination of significance was 0.05. All data are available upon request.

RESULTS

Sex ratios

Regression analyses: Results for first and second sex ratios are shown in Table 1. We analyzed sex ratios of the direct-developing proportion of the brood and of the total brood (direct-developing and diapause individuals).

For the direct first sex ratio, logistic analysis reveals significant parent-offspring regressions for all values of the constant. For the total sex ratio, logistic analysis reveals significant parent-offspring regressions for all but one value of the constant. Mother and daughter direct first sex ratios are shown in Figure 1. Least-squares analysis reveals a significant parent-offspring regression for the direct first sex ratio but not for the total first sex ratio.

For the direct and total second sex ratios, logistic regression analysis reveals significant parent-offspring regressions for all values of the constant. Mother and daughter direct second sex ratios are shown in Figure 2. Least-squares analysis does not detect a significant parent-offspring regression for the direct or the total second sex ratios.

Standard quantitative genetic theory (as in MARGOLIES and COX 1992) implies that the narrow-sense heritability of a trait is twice the value of the parent-offspring regression coefficient, β. This relation stems from the fact

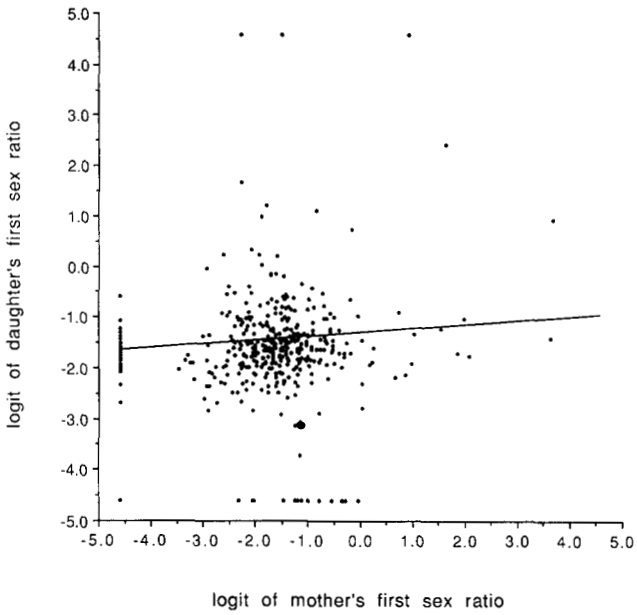

FIGURE 1.—Logistic regression analysis of mother and daughter direct first sex ratios (proportion males + 0.01). All-male sex ratios were scored as 0.99. The solid line depicts the regression equation: logit(daughter sex ratio) = −1.306 + 0.077 × logit(mother sex ratio).

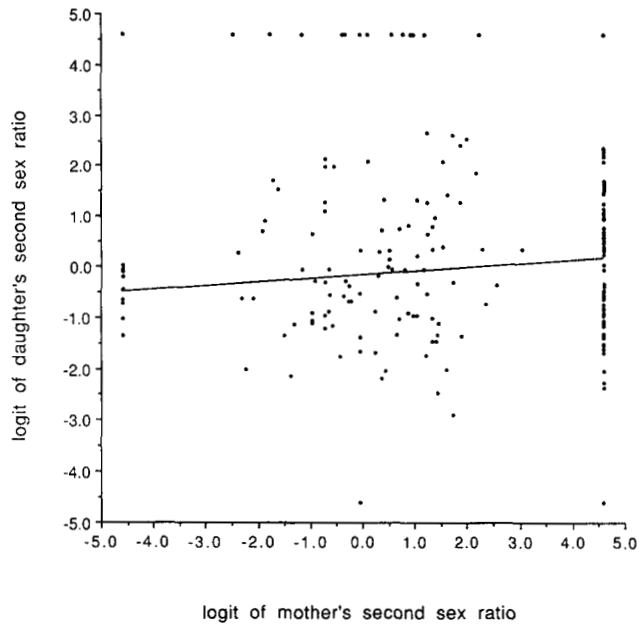

FIGURE 2.—Logistic regression analysis of mother and daughter direct second sex ratios (proportion males − 0.01). All-female sex ratios were scored as 0.01. The solid line depicts the regression equation: logit(daughter sex ratio) = −0.229 + 0.075 × logit(mother sex ratio).

that the numerator of a least-squares estimate of β (the covariance of parent and offspring trait values) is equal to half of the additive genetic variance. When the estimate of β is derived by the method of maximum likelihood or, as in the present case, by the method of quasi-likelihood, it is not expressible as the covariance of parent and offspring values divided by the parental variance [except if the trait is assumed to be normally distributed; see WEDDERBURN (1974) for further details]. As noted above, we assumed that the trait distribution is binomial-like when using quasi-likelihood to estimate the parent-offspring regression and to this extent, doubling of β to estimate the additive genetic variance is an approximation. In any case, the resulting estimates of first sex ratio heritabilities are in general accord with the heritability estimates of ≈0.15 and ≈0.17 derived from analysis of the selection responses of two strains (PARKER and ORZACK 1985). (The heritability estimates in that paper are incorrectly calculated as 1.5 times the regression coefficient; the correct estimates are given here.)

In the context of the estimation of heritability, there is an important point to be made about the use of constants to make the logits of all of the mother sex ratios (and diapause proportions) finite. Such constants are needed only so that mother and daughter traits are expressed on the same scale (as is necessary for a heritability estimate). For what it is worth, logistic regression analyses using the mother's "raw" sex ratio as a predictor revealed significant parent-offspring regressions for the direct and total first and second sex ratios (not shown).

Tests of heterogeneity: In the mother generation, there was a significant effect of the number of days of prefeeding on the first and second sex ratios (first sex ratio: Kruskal-Wallis $H = 47.41$, 3 d.f., $P = 0.0001$, $n = 517$; second sex ratio: $H = 14.34$, 3 d.f., $P = 0.0025$, $n = 242$). In the daughter generation, first sex ratios did not differ significantly across days ($H = 0.09$, 2 d.f., $P = 0.96$, $n = 517$) but second sex ratios did differ ($H = 13.03$, 2 d.f., $P = 0.0015$, $n = 242$). The presence of significant heterogeneity when prefeeding is uniform (as in the daughter generation) implies that uncontrolled environmental variability may have affected the experiment. One consequence is that our heritability estimates may be underestimates.

TABLE 2

Spearman rank correlations (r_s) between first and second sex ratios

	Generation				
	Mother		Daughter		
	r_s	n	r_s	n	χ^2
Direct	0.210[a]	339	0.159[b]	383	0.47
Diapause	0.194	78	0.202	48	0.01
χ^2	0.02		0.08		

All χ^2 values have one degree of freedom. n denotes sample size.
[a] $P < 0.0005$.
[b] $P < 0.002$.

Within-female relationship between first and second sex ratios: Direct first and second sex ratios were significantly correlated in mother and daughter generations (see Table 2). There is no significant difference between the correlations ($\chi^2 = 0.47$, 1 d.f., $P > 0.05$).

TABLE 3

Genetic correlations between direct first and second sex ratios

Constant	r_{12}	SE	C.I.	$C.I._{bc}$	r_{21}	SE	C.I.	$C.I._{bc}$
0.01	0.406	0.324	−0.092 0.910	−0.099 0.910	0.469	0.302	−0.608 1.594	−0.760 1.527
0.001	0.308	0.357	−0.213 0.787	−0.171 0.825	0.463	0.310	−0.765 1.725	−0.998 1.670
0.0001	0.241	0.379	−0.285 0.757	−0.274 0.775	0.458	0.318	−0.597 1.678	−0.665 1.655
0.00001	0.197	0.394	−0.321 0.717	−0.298 0.733	0.456	0.325	−0.807 1.637	−0.830 1.625
None	1.064	−0.072	1.023 1.124	1.011 1.110	0.132	0.534	0.040 0.218	0.038 0.214
n	366				234			

r_{12} denotes the estimate based upon the covariance between the mother's first sex ratio and the daughter's second sex ratio. r_{21} denotes the estimate based upon the covariance between the mother's second sex ratio and the daughter's first sex ratio. S.E. denotes standard error. C.I. denotes the 95% confidence interval estimated by the percentile method. $C.I._{bc}$ denotes the 95% confidence interval estimated by the bias-corrected percentile method. Entries alongside constants stem from logistic regression analysis. Entries alongside "none" stem from least-squares analysis. n denotes the sample size of the covariance estimate.

Diapause first and second sex ratios were not significantly correlated. These analyses should be viewed with caution given the potential dependency of sex ratios within generations (since some broods provided direct and diapause sex ratios) and between generations (as shown above).

As in the estimation of heritabilities, estimation of genetic correlations relies upon the assumption that a logistic regression coefficient is approximately equal to the covariance of parent and offspring logits divided by the variance of parental logits. Estimates of the genetic correlations are shown in Tables 3 and 4. Most of the estimates are smaller than 0.50. The presence of one estimate that is undefined (>1.0) implies that all of the estimates be viewed with caution. It is generally unclear as to how to test for the significance of estimates of genetic correlations. Ignoring the undefined estimate for the moment, in all but one instance (see the bottom of Table 3) there is qualitative agreement between the conclusion about significance based on the standard error and the assumption of a normal sampling distribution and the conclusions based on the two types of bootstrap confidence intervals. There is, however, appreciable quantitative discrepancy between the standard error and bootstrap confidence intervals in many instances, with the general pattern being that the standard error intervals are larger than the bootstrap intervals for r_{12} and smaller for r_{21}. This distinction between r_{12} and r_{21} reflects the lesser variability of first sex ratios (which are used to estimate the covariance in the numerator of r_{12}) relative to the variability of second sex ratios (which are used to estimate the covariance in the numerator of r_{21}). This overall quantitative disagreement between the standard error and bootstrap confidence intervals contrasts with the results of TOWNSEND (1990) who showed that standard error and bootstrap confidence intervals were "reasonably" close for the six genetic correlations examined [see also BROWN (1969) who showed in a Monte Carlo sampling study that sample and true variances were reasonably close for genetic correlations that were small or intermediate in absolute value].

It is relevant in this context to note that almost all of the bootstrap distributions did not differ significantly from normal distributions (see Table 5). Ironically, the only exceptions are those distributions associated with arc-sine transformed proportions. This correspondence is encouraging as it implies that the assumption of a normal sampling distribution can be appropriate for tests of significance even when the estimates of genetic correlations and heritabilities are not "intermediate" in absolute value. (Of course, this presumes that the bootstrap distribution reflects the "true" underlying distribution of the genetic correlation. Such similarity is the fundamental assumption of the bootstrap technique; see BANKS 1989.)

What remains to be answered is the question as to whether there is a genetic correlation between first and second sex ratios. On the one hand, tests based on logistic regression analysis imply that r_{12} and r_{21} do not differ significantly from 0.0 (see Tables 3 and 4). On the other hand, tests based on analysis of arc-sine transformed data lead to the opposite conclusion (except for r_{21} based on the total sex ratio). Given that no scale can be regarded as canonical in a biological sense, the choice as to which analysis has correctly described the biology in question is dependent on external criteria. Although logistic regression analysis is not without problems, for the reasons noted above it is better suited to the analysis of sex ratio data (especially those based on unequal brood sizes) than is least-squares analysis of arc-sine transformed data. To this extent and also given the undefined estimate of r_{21} associated with the arc-sine analysis, it seems reasonable to conclude that there is no strong evidence for significant genetic correlations between first

TABLE 4

Genetic correlations between total first and second sex ratios

Constant	r_{12}	S.E.	C.I.	$C.I._{bc}$	r_{21}	S.E.	C.I.	$C.I._{bc}$
0.01	0.350	0.336	−0.060 0.747	−0.074 0.728	0.421	0.315	−0.789 1.642	−0.843 1.536
0.001	0.317	0.368	−0.143 0.747	−0.171 0.825	0.420	0.336	−0.932 1.770	−1.013 1.746
0.0001	0.281	0.401	−0.124 0.673	−0.135 0.668	0.427	0.357	−1.054 1.864	−1.052 1.866
0.00001	0.252	0.432	−0.209 0.683	−0.234 0.644	0.435	0.374	−1.026 2.012	−1.030 1.955
None	0.733	0.281	0.685 0.786	0.682 0.783	0.059	0.606	−0.028 0.133	−0.020 0.146
n	466				263			

r_{12} denotes the estimate based upon the covariance between the mother's first sex ratio and the daughter's second sex ratio. r_{21} denotes the estimate based upon the covariance between the mother's second sex ratio and the daughter's first sex ratio. S.E. denotes standard error. C.I. denotes the 95% confidence interval estimated by the percentile method. $C.I._{bc}$ denotes the 95% confidence interval estimated by the bias-corrected percentile method. Entries alongside constants stem from logistic regression analysis. Entries alongside "none" stem from least-squares analysis. n denotes the sample size of the covariance estimate.

TABLE 5

Goodness-of-fit tests between the bootstrap distribution associated with the genetic correlation of first and second sex ratios and a normal distribution

	r_{12}(direct)		r_{12}(total)		r_{21}(direct)		r_{21}(total)	
Constant	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.
0.01	15.97	11	8.34	12	17.51	12	10.73	13
0.001	9.16	14	16.65	12	12.67	11	15.51	12
0.0001	11.08	13	11.76	12	11.73	12	19.25	17
0.00001	12.12	11	14.24	12	20.65	13	3.41	11
None	398.6[a]	10	355.8[a]	8	241.9[a]	6	166.7[a]	7

r_{12} and r_{21} are defined as in Tables 3 and 4. Entries alongside constants stem from logistic regression analysis. Entries alongside "none" refer to least-squares regression analysis of arc-sine transformed sex ratios. Expected numbers for each test were calculated using a normal distribution with the mean and variance of the observed bootstrap distribution. For each test, a uniform class width was determined by eye except that terminal classes were lumped so that all expected numbers were greater than or equal to 5.0. The bootstrap sample size is 1000.

[a] $P < 0.00001$.

and second sex ratios. This conclusion is consistent with the observation that wasps selected to produce a less female-biased first sex ratio do not appear to produce altered second sex ratios (ORZACK and PARKER 1986).

Brood sizes

Regression analyses: Results for the size of the broods associated with the first sex ratio and the second sex ratio are shown in Table 6. (Hereafter, these broods are denoted as "first brood" and "second brood".) For both, we analyzed sizes of the direct-developing proportion of the brood and of the total brood. For the first brood and the second brood, there were no significant parent-offspring regressions for both direct and total brood sizes. The sizes for direct and total first broods of daughters were "close" to being normally distributed (not shown). However, the sizes for direct and total second broods were decidedly nonnormal (not shown) and consequently, the results of the regression analyses should be viewed with caution.

Within-female relationship between brood sizes: Sizes of direct first and second broods were significantly correlated in mother and daughter generations (see Table 7). There was a significant difference between the correlations ($\chi^2 = 5.78$, 1 d.f., $P > 0.05$). Sizes of diapause first and second broods were not significantly correlated in either generation. These analyses should be viewed with caution given the dependency of brood sizes within generations (since some broods provide direct and diapause sex ratios).

Diapause proportions

Regression analyses: Results for diapause proportions of first and second sex ratio broods are shown in Table 8. For the first sex ratio, logistic analysis reveals significant parent-offspring regressions for some values of the constant and when a constant is not used. Making any estimate of the heritability of diapause proportion in first-sex-ratio broods is clearly problematic since the estimates of β are negative, implying that an increased diapause proportion in the mother's brood leads to a decreased proportion in the daughter's brood. A possible explanation is presented in the DISCUSSION. For the second sex ratio, logistic and least-squares analyses

TABLE 6

Values of the regression coefficient, β, relating mother and daughter brood sizes

	First-sex-ratio brood				Second-sex-ratio brood			
	Direct		Total		Direct		Total	
	β	χ^2	β	χ^2	β	χ^2	β	χ^2
	-0.036	0.42	0.063	1.60	-0.049	2.00	0.108	2.13
n	396		517		214		278	

Entries stem from least-squares regression analysis. The χ^2-value measures the effect of adding β to the regression model. Each value has one degree of freedom. *n* denotes sample size.

TABLE 7

Spearman rank correlations (r_s) between the brood sizes of first and second sex ratios

	Generation				
	Mother		Daughter		
	r_s	*n*	r_s	*n*	χ^2
Direct	0.187[a]	339	0.358[b]	383	5.78
Diapause	0.057	78	0.018	48	0.04
χ^2	1.01		4.83		

All χ^2 values have one degree of freedom. *n* denotes sample size.
[a] $P < 0.001$.
[b] $P < 0.0005$.

TABLE 8

Values of the regression coefficient, β, relating mother and daughter diapause proportions

	First-sex-ratio brood		Second-sex-ratio brood	
Constant	β	χ^2	β	χ^2
0.01	-0.076	4.92[a]	0.019	0.11
0.001	-0.045	4.06[a]	0.015	0.16
0.0001	-0.032	3.57	0.012	0.17
0.00001	-0.025	3.28	0.010	0.18
None	-0.757	6.03[a]	0.150	0.12
n	517		278	

Entries alongside constants stem from logistic analysis of all pairs of broods. Entries alongside "none" stem from logistic regression analysis in which the mother's "raw" proportion is used as predictor. The χ^2 value measures the effect of adding β to the regression model. Each value has one degree of freedom. *n* denotes sample size.
[a] $P < 0.05$.

revealed no significant parent-offspring regressions for diapause proportions.

Within-female relationship between diapause proportions: Diapause proportions of first and second sex ratio broods were significantly correlated in mother and daughter generations (mother generation: Spearman rank correlation $r_s = 0.604$, $n = 585$, $P < 0.0005$; daughter generation: $r_s = 0.753$, $n = 451$, $P < 0.0005$). These correlations differ significantly ($\chi^2 = 18.78$, 1 d.f., $P < 0.001$).

Effect of the second sex ratio brood on the *stDR* brood

These analyses were motivated by ORZACK and PARKER's (1990) finding that second broods had significant effects on the sex ratios and brood sizes of *stDR* females. We first compared the distributions of *stDR* sex ratios and brood sizes found in singly ("pure") and doubly parasitized ("mixed") hosts (using the combined data from the mother and daughter generations). The distributions of pure and mixed sex ratios did not differ significantly (Kolmogorov-Smirnov $D = 0.091$, pure $n = 322$, mixed $n = 1086$, $P > 0.05$) but the distributions of pure and mixed brood sizes did differ significantly ($D = 0.294$, $P < 0.0005$). The average *stDR* pure brood size was almost 10 individuals larger than the average for mixed broods (45.1 *vs.* 35.7). Thus, there appears to be a negative but non-sex-specific effect of the second brood on the size of the first brood. However, there are three reasons for caution in interpretation. First, both pure and mixed sex ratios and pure and mixed brood sizes differed significantly in the mother generation but not in the daughter generation (mother generation: sex ratios $D = 0.165$, brood sizes $D = 0.226$, pure $n = 280$, mixed $n = 614$, $P < 0.05$; daughter generation: sex ratios $D = 0.163$, brood sizes $D = 0.229$, pure $n = 42$, mixed $n = 472$, $P > 0.05$). Second, a comparison of pure *stDR* broods and control *stDR* broods indicates that the associated distributions of sex ratios did not differ significantly ($D = 0.155$, pure $n = 322$, control $n = 207$, $P > 0.05$) but their distributions of brood sizes did differ significantly ($D = 0.213$, $P < 0.02$). Accordingly, there appear to have been uncontrolled changes in host quality (or less likely, in the reproductive ability of *stDR* females) during the experiment. The final reason for caution in interpretation is that some *stDR* sex ratios and brood sizes may not be independent within each generation since some females were used more than once (see above).

DISCUSSION

Biology of diapause: The basis for the significantly negative parent-offspring regression for diapause tendency in first-sex-ratio broods is unclear. It might reflect a tendency to produce a diapause proportion that is the "opposite" of the proportion in one's natal brood. (Such a between-generation "switch" of phenotypes is not unprecedented, see FALCONER 1960). If present, such a switch might have evolved as a way of protecting offspring against local resource depletion. To the extent

that an ovipositing female is depleting the resources potentially available to her offspring (since her prey cannot reproduce), it would be advantageous for a direct-developing female to delay her offspring's emergence by putting them into diapause until resource levels have rebounded. It would also be advantageous for a diapause female to *not* delay her offspring's emergence (since her mode of development implies that resources have not been recently depleted). Of course, it is not clear that wasp parasitization controls fly abundance strongly enough that switching ability could evolve especially if most hosts have appreciable proportions of both types of larvae (although in this experiment, most hosts contained only one type). In addition, it is likely that the main determinant of diapause evolution in the ancestral population of the isofemale strains used here is the annual change in temperature associated with the beginning and end of the "growing season." Of course, it is possible that a switch mechanism could evolve as a response to this change. To the extent that a diapause female "thinks" that she has emerged in the spring, it would be advantageous for her to produce direct-developing offspring since they will reproduce more quickly. To the extent that an direct-developing female "thinks" she has emerged late in the season, it is advantageous for her to produce diapause offspring so that her lineage will survive the winter. What remains uncertain about this argument is the number of "live" generations per growing season in this locality and more importantly, whether the diapause behavior observed under the experimental temperature and light regime (25° and 24 hr light) is an aberrant response to these "unnatural" conditions or is at least partially reflective of the natural behavior. Further experiments are clearly needed.

Nature of genetic variation: In a basic sense, this experiment was an attempt to measure parent-offspring resemblance. It should not be thought of as an attempt to measure narrow-sense heritability if such an endeavor is taken to imply that one take seriously the standard quantitative genetic model of gene action (*cf.* FALCONER 1989). Beyond philosophical concerns, this viewpoint is motivated by the descriptions of non-Mendelian "factors" that affect sex ratios in this species (SAUL 1961; SKINNER 1982; 1985; WERREN *et al.* 1981). While there is no evidence that such factors affected the sex ratios measured in this experiment (and the presence of at least some types of these factors is quite unlikely for a number of reasons), the possibility that they could be present serves as a reminder that the significant heritabilities described in this study need not simply reflect the Mendelian segregation of alleles at "standard" loci.

Evolutionary significance of these results: Whether these results are of relevance to our understanding of sex ratio evolution depends upon the validity of the assumption that the wasps emerging at any one time from a bird nest (or similar small area) actually constitute a breeding population, *i.e.*, that individuals from a given nest mate among themselves. Even crude genetic and behavioral data relating to this issue are almost completely lacking for parasitoids (but see KAZMER and LUCK 1991; NADEL and LUCK 1992) [see also HARDY (1994) for related discussion]. R. MADEJ and S. SKINNER (personal communication) have shown in a laboratory study of *N. vitripennis* that a large proportion of matings can occur among host-mates. Even if one presumes that such mating behavior is usual, what is unclear is whether such "locally" mated females disperse at random or whether they reproduce in a local group. It could well be that individuals disperse in nature in such a way that local aggregations of wasps exhibit no significant heritabilities for these traits. Resolution of this question depends upon the acquisition of (1) data on the heritabilities in populations derived from other localities, (2) genetic data on the population structure of this species and (3) data on the mating and dispersal behaviors of females and males in nature.

Despite the absence of data concerning population structure and breeding biology, it is still striking that individuals sampled from such a small area give rise to populations with significant heritabilities for both of the sex ratio traits. This was contrary to our expectations and to those of some of our colleagues. After all, the original individuals sampled from the bird nest may easily have been the offspring of one or two females. It is compelling in this regard that we detected no significant heritabilities for first and second brood sizes. The distinction between this finding and the sex ratio finding indicates that the ancestral population may not have been "inherently" variable in the way that evolutionary biologists often presume populations to be. At least tentatively, the distinction can be attributed to the different ways in which natural selection may act on these traits. There may be directional selection on brood size such that the genetic variation for this trait has been exhausted. In contrast, the inherent frequency-dependence of sex ratio evolution in multiple-foundress populations implies that there is a potential for genetic variation to be maintained in natural populations. The tentative nature of this explanation is underscored by the fact that RAM and SHARMA (1977) detected genetic variation for brood size but *not* for sex ratio in another parasitoid (see also WAJNBERG *et al.* 1989).

There is an important contrast to be made in regard to understanding the meaning of our results. Consider the contrast between our results and other data on genetic variation in Hymenoptera. In particular, Hymenoptera are *less* variable on the average at soluble enzyme loci than are diplodiploid insects (see AVERY 1984; GRAUR 1985). Yet if this study and previous studies (ORZACK and PARKER 1990; ORZACK *et al.* 1991) have revealed typical results for Hymenoptera, this group has

more genetic variation for sex ratio traits, as there are no reports of heritable variation for sex ratio traits in species of Diptera, for example. [TORO and CHARLESWORTH (1982) provide further details]. If anything, this contrast makes clear that the presence of genetic variation for sex ratio traits should not be regarded as a "given" simply because sex ratios in this species are determined partially by female behavior and are not determined by the segregation of sex chromosomes. (On the other hand, the absence of such variation in diplodiploid insects should not be regarded as a given; few studies are as thorough as TORO and CHARLESWORTH's and even they studied flies from only one area.)

There is another motivation for further investigations of whether local populations of Hymenoptera have genetic variation for sex ratio traits: such investigations can be part of the assessment of the validity of the common claim that sex ratio traits in these species are locally optimal in the evolutionarily stable strategy (ESS) sense. By definition, an ESS is a trait that, when fixed or nearly fixed in a population, prevents any other trait from entering [see HINES (1987) for further details]. To this extent, the claim that an ESS has evolved requires an assessment of phenotypic variation and the determination of (1) whether such variation results in significant between-individual (or between-isofemale-strain) variation in the *quantitative* fit of the trait to the prediction of the model and (2) whether the model is quantitatively accurate. If such heterogeneity of fit is not present and the model is accurate, it means that an ESS has evolved. In *practice*, this is an achievable claim (see ORZACK and SOBER 1994). If such heterogeneity of fit is present and the associated phenotypic differences are heritable, it may reflect the fact that "genetics has gotten in the way" of the evolution of a locally optimal phenotype (the ESS). Either kind of test result contributes to an ensemble test of adaptationism [see ORZACK and SOBER (1994) for further details].

Our data are also relevant to an important ambiguity in present understanding of the basis for sex ratio evolution in this species. There is a potential conflict between the presence of genetic variation for sex ratio traits within local populations of this species and the commonly published conclusion that it has a subdivided population structure (*e.g.*, see WERREN 1983). Present theory implies that extensive polymorphism for a sex ratio trait is more likely in a large, panmictic population (*e.g.*, all sex ratios are selectively neutral with respect to one another in an infinite panmictic population, see KOLMAN 1960). Taken at face value, the conclusion that there is population subdivision in this species is plausible. Such a population structure implies that local breeding groups should have little genetic variation for sex ratio traits if their evolution is driven only by natural selection (since selection for the optimal phenotype will be strongest under these conditions, see HINES 1982). The problem is that this conclusion has not been accompanied by any behavioral, ecological, or genetic *data* although, as noted above, the data of MADEJ and SKINNER are at least partially supportive of this conclusion and it is clear that such population subdivision could underlie the evolution of the female-biased sex ratios often produced by females of this species [see HAMILTON (1967), HERRE (1985), and FRANK (1986) for further details]. Nonetheless, the standard ecological and morphological arguments used to motivate the conclusion that there "must" be population subdivision in this species are ambiguous for several reasons (ORZACK 1992). Even if they were compelling, such arguments are not directly relevant, as present theory indicates that it is *genetic* subdivision (defined in the standard Wrightian sense) and not *ecological* subdivision that is a prerequisite for the evolution of a female-biased sex ratio (see FRANK 1986). Whether there is a real conflict between the data on polymorphism reported in this study and previous studies and the conclusion that this species has population subdivision can be resolved only when we have better and more extensive genetic data on variation for sex ratio traits and on the nature of population structure.

We thank L. MAGNI for collecting the bird nest from which the original wasps were collected, P. MCCULLAGH for statistical advice, D. PARKER for graciously supplying the isofemale strains, D. RIVERS for supplying hosts, P. SNIEGOWSKI for help in the laboratory, and H. AKASHI, J. BULL, R. CHAPPELL, J. CROW, A. GIMELFARB, M. LYNCH, D. PARKER, R. SHAW, S. SKINNER, K. SMITH and an anonymous reviewer for assistance or comments. S.H.O. thanks B. CHARLESWORTH and M. KREITMAN for logistical and financial support.

LITERATURE CITED

ANTOLIN, M. F., 1992a Sex ratio variation in a parasitic wasp. I. Reaction norms. Evolution **46:** 1496–1510.

ANTOLIN, M. F., 1992b Sex ratio variation in a parasitic wasp. II. Diallel cross. Evolution **46:** 1511–1524.

AVERY, P. J., 1984 The population genetics of haplo-diploids and X-linked genes. Genet. Res. **44:** 321–341.

BANKS, D. L., 1989 Bootstrapping–II, pp. 17–22 in *Encyclopedia of Statistical Sciences* (supplement volume), edited by S. KOTZ and N. L. JOHNSON. John Wiley & Sons, New York.

BECKER, W., 1984 *Manual of Quantitative Genetics.* Academic Enterprises, Pullman, Wash.

BROWN, G. H., 1969 An empirical study of the distribution of the sample genetic correlation coefficient. Biometrics **25:** 63–72.

CROSBIE, S. F., and G. N. HINCH, 1985 An intuitive explanation of generalized linear models. N.Z. J. Agric. Res. **28:** 19–29.

DAS, L. K., 1959 Studies on the comparative longevity, fecundity and sex ratio of some races of *Trichogramma evanescens minutum* Riley and their reciprocal crosses. Associateship Thesis, Indian Agricultural Research Institute, New Delhi.

EFRON, B., and R. TIBSHIRANI, 1986 Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. Stat. Sci. **1:** 54–77.

FALCONER, D. S., 1960 The genetics of litter size in mice. J. Cell. Comp. Physiol. **56** (Suppl. 1): 153–167.

FALCONER, D. S., 1989 *Introduction to Quantitative Genetics.* John Wiley & Sons, New York.

FRANK, S. A., 1986 Hierarchical selection theory and sex ratios. I. General solutions for structured populations. Theor. Popul. Biol. **29:** 312–342.

GRAUR, D., 1985 Gene diversity in Hymenoptera. Evolution **39:** 190–199.

HAMILTON, W. D., 1967 Extraordinary sex ratios. Science **156:** 477–488.

HARDY, I. C. W., 1994 Sex ratio and mating structure in the parasitoid Hymenoptera. Oikos **69:** 3–20.

HAWKES, P. G., 1992 Sex ratio stability and male-female conflict over sex ratio control in hymenopteran parasitoids. S. Afr. J. Sci. **88:** 423–430.

HERRE, E. A., 1985 Sex ratio adjustment in fig wasps. Science **228:** 896–898.

HINES, W. G. S., 1982 Mutations, perturbations and evolutionarily stable strategies. J. Appl. Prob. **17:** 600–610.

HINES, W. G. S., 1987 Evolutionarily stable strategies: a review of basic theory. Theor. Popul. Biol. **31:** 195–272.

JANZEN, F. J., and G. L. PAUKSTIS, 1991 Environmental sex determination in reptiles: ecology, evolution, and experimental design. Q. Rev. Biol. **66:** 149–179.

KAZMER, D. J., and R. F. LUCK, 1991 The genetic-mating structure of natural and agricultural populations of *Trichogramma*, pp. 107–110 in *Trichogramma and Other Egg Parasitoids*, edited by E. WAJNBERG and S. B. VINSON. INRA, Paris.

KOLMAN, W., 1960 The mechanism of natural selection for the sex ratio. Am. Nat. **94:** 373–377.

LEGNER, E. F., 1988 *Muscidifurax raptorellus* (*Hymenoptera: Pteromalidae*) females exhibit postmating oviposition behavior typical of the male genome. Ann. Entomol. Soc. Am. **81:** 522–527.

LEGNER, E. F., 1989 Paternal influences in males of *Muscidifurax raptorellus* (*Hymenoptera: Pteromalidae*). Entomophaga **34:** 307–320.

MARGOLIES, D. C., and T. S. COX, 1992 Quantitative genetics applied to haplodiploid insects and mites, pp. 548–559 in *Evolution and Diversity of Sex ratio in Insects and Mites*, edited by D. L. WRENSCH and M. A. EBBERT. Chapman & Hall, New York.

MCCULLAGH, P., and J. A. NELDER, 1989 *Generalized Linear Models*. Chapman & Hall, New York.

NADEL, H., and R. F. LUCK, 1992 Dispersal and mating structure of a parasitoid with a female-biased sex ratio: implications for theory. Evol. Ecol. **6:** 270–278.

ORZACK, S. H., 1990 The comparative biology of second sex ratio evolution within a natural population of a parasitic wasp, *Nasonia vitripennis*. Genetics **124:** 385–396.

ORZACK, S. H., 1992 Sex ratio evolution in parasitic wasps, pp. 477–511 in *Evolution and Diversity of Sex Ratio in Insects and Mites*, edited by D. L. WRENSCH and M. A. EBBERT. Chapman & Hall, New York.

ORZACK, S. H., and E. D. PARKER, JR., 1986 Sex-ratio control in a parasitic wasp, *Nasonia vitripennis*. I. Genetic variation in facultative sex-ratio adjustment. Evolution **40:** 331–340.

ORZACK, S. H., and E. D. PARKER, JR., 1990 Genetic variation for sex ratio traits within a natural population of a parasitic wasp, *Nasonia vitripennis*. Genetics **124:** 373–384.

ORZACK, S. H., and E. SOBER, 1994 Optimality models and the test of adaptationism. Am. Nat. **143:** 361–380.

ORZACK, S. H., E. D. PARKER, JR., and J. GLADSTONE, 1991 The comparative biology of genetic variation for conditional sex ratio adjustment in a parasitic wasp, *Nasonia vitripennis*. Genetics **127:** 583–599.

PARKER, E. D., JR., and S. H. ORZACK, 1985 Genetic variation for the sex ratio in *Nasonia vitripennis*. Genetics **110:** 93–105.

RAM, A., and A. K. SHARMA, 1977 Selective breeding for improving the fecundity and sex-ratio of *Trichogramma fasciatum* (Perkins) (Trichogrammatidae: Hymenoptera), an egg parasite of lepidopterous hosts. Entomon **2:** 133–137.

SASTRY, K. S. S., 1962 Studies on the behaviour of some races of *Trichogramma evanescens minutum* Riley and their crosses. Master's Thesis, Indian Agricultural Research Institute, New Delhi.

SAUL, G. B., 1961 An analysis of non-reciprocal cross incompatibility in *Mormoniella vitripennis* (Walker). Z. Vererbungsl. **92:** 28–33.

SIMMONDS, F. J., 1947 Improvement of the sex-ratio of a parasite by selection. Can. Entomol. **79:** 41–44.

SKINNER, S. W., 1982 Maternally inherited sex ratio in the parasitoid wasp, *Nasonia vitripennis*. Science **215:** 1133–1134.

SKINNER, S. W., 1985 Son-killer: a third extrachromosomal factor affecting the sex ratio in the parasitoid wasp, *Nasonia* (=*Mormoniella*) *vitripennis*. Genetics **109:** 745–759.

THOMPSON, R., 1990 Generalized linear models and applications to animal breeding, pp. 312–328 in *Advances in Statistical Methods for Genetic Improvement of Livestock*, edited by D. GIANOLA and K. HAMMOND. Springer-Verlag, New York.

TORO, M. A., and B. CHARLESWORTH, 1982 An attempt to detect genetic variation in sex ratio in *Drosophila melanogaster*. Heredity **49:** 199–209.

TOWNSEND, D. R., 1990 The genetic basis of a complex phenotype. Ph.D. Thesis, University of Chicago.

WAJNBERG, E., J. PIZZOL and M. BABAULT, 1989 Genetic variation in progeny allocation in *Trichogramma maidis*. Entomol. Exp. Appl. **53:** 177–187.

WEDDERBURN, R. W. M., 1974 Quasi-likelihood functions, generalized linear models, and the Gauss-Newton method. Biometrika **61:** 439–447.

WERREN, J. H., 1983 Sex ratio evolution under local mate competition in a parasitic wasp. Evolution **37:** 116–124.

WERREN, J. H., S. W. SKINNER and E. L. CHARNOV, 1981 Paternal inheritance of a daughterless sex ratio factor. Nature **293:** 467–468.

WILKES, A., 1947 The effects of selective breeding on the laboratory propagation of insect parasites. Proc. R. Soc. Lond. Ser. B **134:** 227–245.

WRENSCH, D. L., and M. A. EBBERT, 1992 *Evolution and Diversity of Sex Ratio in Insects and Mites*. Chapman & Hall, New York.

Communicating editor: M. LYNCH