Haplodiploidy and the Evolution of Facultative Sex Ratios in a Primitively Eusocial Bee

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In eusocial Hymenoptera, the haplodiploid system of sex determination creates relatedness asymmetries such that workers are more closely related on average to their sisters than to their brothers. For such societies, kin-selection theory and sex-ratio theory predict that workers maximize their inclusive fitness by biasing the investment sex ratio toward females. To test the prediction of sex-ratio biasing, relatedness asymmetries were experimentally manipulated in colonies of the primitively eusocial bee *Augochlorella striata* (Halictidae: Hymenoptera) by removing or not removing foundress queens. Queenright colonies (relatedness asymmetry present) produced a more female-biased sex ratio than did queenless colonies (relatedness asymmetry absent). Worker reproduction and unmated replacement queens can be discounted as alternative explanations. Workers therefore facultatively adjusted their colony's sex ratio and, in the presence of a relatedness asymmetry, biased the investment sex ratio toward their more closely related sisters and away from their more distantly related brothers.

I N HYMENOPTERAN EUSOCIAL SOCIE-ties, the haplo-diploid system of sex determination creates relatedness asymmetries between workers and the male and female sibling reproductives (sexuals) that the workers help to produce (1–4). In particular, workers are more closely related on average to their sisters than to their brothers. This difference in relatedness to sisters versus brothers measures the extent of the relatedness asymmetry. Unifying sex-ratio theory (5) and kin-selection theory (1, 2), Trivers and Hare (4) hypothesized that relatedness asymmetries in colonies of eusocial Hymenoptera should selectively favor workers, biasing the investment sex ratio toward females (6), thus creating a conflict over the colony's sex ratio between workers and their mother (the queen). The conflict between the mother and her daughter workers derives from the fact that the mother is symmetrically related to her male and female offspring (both $r = 1/2$), whereas workers are asymmetrically related to their brothers ($r = 1/4$) and sisters ($r = 1/4 + 1/2n$; $n =$ number of matings by the mother) and therefore prefer a relatively more female-biased sex ratio than does their mother. Under Trivers and Hare's hypothesis, if workers are at least in partial control of the investment sex ratio, then workers are predicted to bias the sex ratio away from the sex ratio preferred by the mother. Concordant with previous nonexperimental studies (4, 7), I present experimental evidence supporting Trivers and Hare's hypothesis by demonstrating that relatedness asymmetries in a eusocial hymenopteran society cause workers to invest preferentially in sisters as opposed to brothers.

Boomsma (8) recently outlined an experimental approach to test whether workers in hymenopteran societies bias the sex ratio toward females. Expanding on the original work by Trivers and Hare (4) and on recent work by others (9, 10), Boomsma (8) predicted facultative, intrapopulation differences in sex ratios between eusocial colonies (mother-daughter associations) and parasocial colonies (sister-sister associations resulting from the loss of the mother). Only in eusocial colonies, that is, in two-generation colonies with a reproducing mother, can workers capitalize on relatedness asymmetries by biasing the sex ratio toward females. No such asymmetry exists in the single-generation, parasocial colonies, regardless of whether a female herself reproduces, or one of her sisters. Workers in eusocial colonies are therefore predicted to favor a more female-biased sex ratio as compared to the sex ratio favored by workers in parasocial colonies.

Furthermore, if workers in eusocial colonies succeed at biasing the sex ratio toward females, and if eusocial colonies outnumber parasocial colonies, then the resulting shift of the population sex ratio will create a male shortage as seen from the perspective of workers in parasocial colonies (4, 9). To counterbalance the sex ratio shift induced by eusocial colonies, the optimal investment sex ratio for parasocial colonies becomes an exclusive (or almost exclusive) investment in males. The population as a whole therefore moves toward a split sex ratio (9, 11). Consequently, the difference in investment sex ratio between "biasing" eusocial and "balancing" parasocial colonies is augmented, and thus may be relatively easy to demonstrate empirically. Previous nonexperimental studies comparing sex ratios of eusocial and parasocial colonies indicated such split sex ratios in a ponerine ant (12) and a halictine bee (13), but did not reveal differences in sex ratios in a polistine wasp (10).

Field experiments conducted in the summer of 1990 on the primitively eusocial sweat bee *Augochlorella striata* (14) confirmed the predicted sex-ratio difference between eusocial and parasocial colonies. In a paired experimental design (15), 38 monogynous colonies from two nest aggregations (16) were randomly assigned at the beginning of worker emergence to either a parasocial condition (nest foundress removed) or a eusocial condition (nest foundress remained). Foundresses were captured at nest entrances while they guarded their nests. Upon loss of the foundress, one of the daughters assumed the role of the colony's primary reproductive and guard. (Under natural conditions, such supersedure of foundresses occurs in 20 to 40% of all colonies.) One randomly selected worker was removed from all eusocial nests to equalize colony sizes between the two nest conditions (17). Between 21 and 24 days (mean, 22 days) after foundress removal, all nest pairs were excavated, and the brood in intact combs was reared to adulthood in the laboratory (18). Of the sexuals emerging in the laboratory, only those sexuals were included in the analysis that developed from cells provisioned during the respective 21 to 24 days after foundress removal, based on a developmental time from egg to adult of 30 days (19).

Both the numerical sex ratio ($SR_n = \%$ males) and the investment sex ratio ($SR_i = \%$ male wet weight) were significantly more female-biased in the 19 eusocial nests ($SR_n = 49.9\%$, $SR_i = 43.4\%$) than in the 19 parasocial nests ($SR_n = 67.2\%$, $t = 2.02$, $df = 18$, $P = 0.029$; $SR_i = 62.6\%$, $t = 2.08$, $df = 18$, $P = 0.026$; Table 1) (20). In five nests in the eusocial condition, the foundress died during the experiment and one of her daughters superseded in assuming the role of the primary reproductive. These nests therefore began as eusocial nests, superseded, and continued as parasocial nests. Under the hypothesis of facultative sex-ratio adjustments by workers, superseded nests are therefore expected to produce relatively more male-biased sex ratios compared to nests that remained eusocial throughout the duration of the experiment. Indeed, the average sex ratio of the five superseded nests ($SR_n = 77.7\%$, $SR_i = 71.6\%$) was significantly more male-biased than the average sex ratio of the 14 eusocial nests ($SR_n = 40.0\%$, $t = 4.21$, $df = 17$, $P = 0.0003$; $SR_i = 33.3\%$, $t = 3.94$, $df = 17$, $P = 0.0005$). As a consequence, therefore, naturally occurring supersedure confounded the estimate of

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the sex-ratio differences between eusocial and parasocial nests. When excluding the five nest pairs with superseded nests, the remaining 14 eusocial nests showed a substantial female-bias ($SR_n = 40.0\%$, $SR_i = 33.3\%$) as compared to the 14 paired parasocial nests ($SR_n = 73.4\%$, $t = 4.33$, $df = 13$, $P = 0.0004$; $SR_i = 69.0$; $t = 4.08$, $df = 13$, $P = 0.0007$). The results therefore confirm the theoretical predictions derived from kin-selection and sex-ratio theory (4, 8, 9) and replicate experiments conducted during the summer of 1989 (21).

There exist two hypotheses alternative to the relatedness asymmetry hypothesis which also could explain the observed sex-ratio differences. A relatively more male-biased sex ratio in parasocial colonies may have been caused by (i) unmated replacement reproductives in parasocial colonies or (ii) greater worker reproduction of sons in parasocial than in eusocial colonies. To address these two alternative hypotheses, 28 experimental pairs were excavated at 3-day intervals after foundress removal (3 and 6 days, $n = 8$ each; 9, 12, and 15 days, $n = 4$ each), in addition to the 19 experimental pairs excavated at the end of the study (21 to 24 days after foundress removal). All females present in these nests at the time of excavation were dissected and examined for ovarian development and for mating status (presence of sperm in the spermatheca).

Unmated replacement reproductives. If replacement reproductives in parasocial nests were not inseminated, then they would have been unable to produce daughters and would have been forced to produce a male-biased sex ratio (22). Of the 47 parasocial nests examined, only four (two excavated at 6 days after foundress removal and two at 22 days) were headed by a replacement reproductive that was not inseminated (23). All other replacement reproductives of parasocial nests were inseminated and therefore capable of producing daughters. Thus, only a small proportion of parasocial nests (8.5%) was incapable of producing females. When omitting the nest pairs (6 and 9 in Table 1) with uninseminated replacement reproductives from the analysis of the 14 nest pairs excavated at the end of the study, the reduced sample of eusocial nests still shows significantly more female-biased sex ratios ($SR_n = 39.1\%$; $SR_i = 32.8\%$) than the sample of paired parasocial nests ($SR_n = 69.0\%$, $t = 3.82$, $df = 11$, $P = 0.0014$; $SR_i = 66.0\%$, $t = 3.68$, $df = 11$, $P = 0.0018$). This eliminates the unmated condition of replacement reproductives as a complete explanation of the observed sex-ratio biases.

Worker reproduction. The occurrence of workers with ovarian development in both eusocial and parasocial nests suggests a second alternative hypotheses. While a foundress may be able to dominate her daughters and thus prevent worker reproduction of sons because of her size and age advantage, a replacement reproductive may be incapable of preventing her sister workers from occasional production of sons because some of her sister workers may exceed her in size or may be of greater age. Of the 47 parasocial nests excavated, however, only three nests (excavated at 9, 12, and 22 days after foundress removal) contained a worker with a fully developed egg in her ovaries. The maximum proportion of sexuals produced by reproducing workers therefore was less than 7%, with an upper, one-sided, 95% confidence limit of no more than 13% (24). When correcting the observed sex ratios of parasocial nests by 13% worker-produced sons (25), the sex ratios of the 12 eusocial nests ($SR_n = 39.1\%$; $SR_i = 32.8\%$) remain significantly more female-biased than the corrected sex ratios of the paired parasocial nests ($SR_n = 55.02\%$, $t = 2.06$, $df = 11$, $P = 0.032$; $SR_i = 50.72\%$, $t = 2.37$, $df = 11$, $P = 0.019$). This eliminates worker reproduction of sons as a complete explanation of the observed sex-ratio biases.

Because both the unmated condition of replacement reproductives and worker-produced sons can be discounted as alternative explanations, relatedness asymmetries caused by the haplodiploid system of sex determination remain a viable explanation of the observed sex-ratio difference between eusocial and parasocial colonies in *A. striata.* This implies that (i) workers in *A. striata* are at least in partial control of their colony's sex-ratio; (ii) workers perceive cues to the likely presence or absence of relatedness asymmetries in their colony (for example, presence or absence of the foundress); (iii) workers in eusocial nests bias the investment sex ratio toward females (26) as predicted by Trivers and Hare (4); and (iv) variation in relatedness asymmetry among colonies produces a split sex ratio at the level of the entire population (8, 9). The present study therefore provides experimental evidence that relatedness asymmetries in a eusocial hymenopteran society cause workers to invest preferentially in sisters as opposed to brothers. Altruistic behavior in workers of *A. striata* therefore seems to be modulated by relatedness asymmetries intrinsic to the haplodiploid system of sex determination (27).

Table 1. The number of male and female sexuals reared, the numerical sex ratio (SR_n), and the investment sex ratio (SR_i) for each of the 19 experimental nest pairs. Eusocial nests of pairs 1 through 14 retained the nest foundress until the end of the experiment. Eusocial nests of pairs 15 through 19 naturally lost their foundresses sometime during the experiment, showed supersedure by a daughter replacement reproductive, and thus formed parasocial societies by the end of the experiment. Overall, nests in the eusocial condition produced significantly more female-biased sex ratios than nests in the parasocial condition ($P < 0.05$ for both the SR_n and the SR_i). Replacement reproductives in the parasocial nests 6 and 9, and in the supersedure nest in pair 15 were not inseminated; these nests therefore produced a 100% male sexual brood.

Nest pair	Eusocial				Parasocial			
	Females (no.)	Males (no.)	SR_n (% male)	SR_i (% male)	Females (no.)	Males (no.)	SR_n (% male)	SR_i (% male)
1	10	2	16.7	9.9	1	6	85.7	81.6
2	4	5	55.6	50.1	6	6	50.0	43.5
3	7	3	30.0	24.4	1	3	75.0	72.6
4	7	4	36.4	27.2	3	8	72.7	69.9
5	3	5	62.5	50.5	2	7	77.8	74.1
6	5	8	61.5	56.4	0	9	100.0	100.0
7	11	3	21.3	14.8	4	4	50.0	42.3
8	2	4	66.7	61.9	1	1	50.0	33.3
9	7	3	30.0	22.8	0	11	100.0	100.0
10	7	8	53.3	44.3	4	9	69.2	63.4
11	15	3	16.7	10.3	1	9	90.0	92.7
12	9	6	40.0	34.9	2	10	83.3	82.1
13	5	4	44.4	38.7	2	8	80.0	71.7
14	6	2	25.0	20.2	5	4	44.4	39.1
15	0	7	100.0	100.0	2	3	60.0	52.7
16	2	7	77.8	69.5	6	4	40.0	37.8
17	1	3	75.0	65.7	1	5	83.3	79.4
18	1	6	85.7	83.6	6	5	45.5	36.6
19	3	3	50.0	39.4	4	1	20.0	16.8
Mean (SD)			49.9 (24.23)	43.4 (25.09)			67.2 (22.11)	62.6 (24.46)

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14. *Augochlorella striata* (Hymenoptera: Halictidae) is a soil-nesting sweat bee with an annual colony cycle divided into three phases: foundress, worker, and reproductive phases [E. Ordway, *Insectes Soc.* 12, 291 (1965)]. In the foundress phase, foundresses initiate nest construction in spring after emergence from winter hibernacula and begin mass-provisioning of their first brood. Cells are constructed of soil and arranged in a cluster (comb) that is suspended in a small subterranean cavity. The first brood consists of both males and females at about equal proportions. The emergence of the first daughters in early summer marks the beginning of the worker phase. The foundress now functions as the colony's primary reproductive (queen) and guard, while daughters function as workers, constructing and provisioning cells. In upstate New York, virtually all nests are founded monogynously, and colony size is about two to eight females in the worker phase. All females cooperate in the production of a second brood of about 5 to 30 reproductives (sexuals). Sexuals emerge in late summer (reproductive phase) and mate, but only female sexuals hibernate and found colonies during the following spring. In 20 to 40% of all colonies, the foundress dies during the worker phase and one of her daughters becomes the primary reproductive (replacement reproductive) of the orphaned, single-generation (parasocial) colony. Because first brood males are present throughout the worker phase, replacement reproductives and workers are able to mate.
15. Pairs of nests were matched for spatial proximity (<75-cm distance) and for day of emergence of the first worker to control for microclimatic and temporal differences among nests.
16. The two study aggregations were located on the embankments of two ponds on the campus of Cornell University (Ithaca, NY) and were separated by about 45 m.
17. Experimental equilibration of colony sizes was only partially successful, because the average colony size of eusocial colonies (4.93 ± 1.63 females, including the foundress) exceeded the average colony size of parasocial colonies (4.50 ± 1.35 females), though this difference was not statistically significant. Ignoring colony type, the correlation between colony size and investment sex ratio was not statistically significant. Investment sex ratio was negatively correlated to colony size in eusocial colonies ($r = 0.51$, $P = 0.092$), but positively correlated in parasocial colonies ($r = 0.28$, $P = 0.31$).
18. Provisioning of cells in earthen combs allows for the excavation of combs in toto, which can then be maintained in the laboratory under controled conditions until brood emergence with minimal mortality. In this way, even eggs laid the day before excavation can be reared to adulthood. Overall, egg, larval, and pupal death in the laboratory was 11.4% (9.1% in eusocial and 14.3% in parasocial colonies). Brood loss due to rhipiphorid parasites amounted to 6.0% (6.3% in eusocial and 5.5% in parasocial colonies).
19. E. Ordway [*Insectes Soc.* 12, 291 (1965); *J. Kans. Entomol. Soc.* 39, 270 (1966)] estimated developmental times for *A. striata* of 28 to 30 days under field conditions. This estimate agrees with my estimate of 29 to 30 days under laboratory conditions.
20. Average sex ratios were compared in one-tailed paired t tests after arcsine transformation of percent male production.
21. The preliminary experiment conducted in 1989 followed a two-sample design with 13 and 15 nests in the parasocial and eusocial condition, respectively [U. G. Mueller, in *Social Insects and the Environment*, G. K. Veeresh, B. Mallik, C. A. Viraktamath, Eds. (Oxford Univ. Press, Oxford, 1990), abstr., p. 363]. Nine of the 15 eusocial nests had lost the foundress by the time of excavation, subdividing this sample into a supersedure condition ($n = 9$) and a truly eusocial condition ($n = 6$). Both the numerical sex ratio and the investment sex ratio were significantly more female-biased in the eusocial nests ($SR_n = 33.6\%$, $SR_i = 26.4\%$) than in the parasocial nests ($SR_n = 57.9\%$, $t = 2.29$, $df = 19$, $P < 0.02$) and also more female-biased than in the supersedure nests ($SR_n = 52.2\%$, $t = 2.10$, $df = 22$, $P < 0.03$; $SR_i = 44.8\%$, $t = 2.31$, $df = 22$, $P < 0.02$).
22. It is not clear, a priori, whether unmatedness of replacement reproductives functions as a mechanistic constraint altering sex ratios in parasocial nests, or alternatively, whether unmatedness is the proximate mechanism of replacement reproductives opting for a strategy of producing a male-biased sex ratio. The latter interpretation would be consistent with the hypothesis of facultative sex-ratio adjustment. Unmatedness of replacement reproductives may therefore not represent a truly alternative explanation.
23. The major reproductive in a nest was identified as the bee that functioned as a guard, but not as a forager, and possessed the most developed ovaries among all females present.
24. Confidence limits were calculated under the assumption that the probability of worker reproduction follows a binomial distribution with an average frequency of 7%.
25. The calculation used to correct for worker reproduction of sons was based on the following assumptions: (i) all of the 13% worker-produced sexuals were male, (ii) the primary reproductive would have produced a female which was replaced by a worker's son, and (iii) workers in eusocial nests do not reproduce. The correction yields an overestimate of the actual extent of worker reproduction and thus maximally biases the test in favor of the hypothesis that worker reproduction of sons caused the male-biased sex ratios in the parasocial nests. The assumptions underlying the correction are overly stringent in that (i) workers may produce not only sons, (ii) the primary reproductive may have produced a male that was replaced by a workers' son, and (iii) workers in eusocial nests are capable of producing sons as indicated by their ovarian development. All of these will reduce the confounding effect of worker-produced sons.
26. The mechanisms of sex-ratio biasing by workers may be (i) direct (killing of male eggs, larvae, or pupae; adding provisionings to cells with female larvae), or (ii) indirect (increasing the queen's probability of laying a female egg by increasing the size of the provisioning mass) (8).
27. The demonstrated importance of relatedness asymmetries in modulating altruistic worker behavior does not imply that relatedness asymmetries (that is, haplodiploidy) played a significant role in the evolution toward eusociality. Extrinsic factors such as parasite and predation pressures may have sufficed to favor eusociality over solitary reproduction or over other forms of sociality [H. E. Evans, *BioScience* 27, 613 (1977); S. T. Emlen, *Am. Nat.* 119, 29 (1982); R. D. Alexander, K. Noonan, B. J. Crespi, in *The Biology of the Naked Mole-Rat*, P. W. Sherman, J. Jarvis, R. D. Alexander, Eds. (Princeton Univ. Press, Princeton, 1991), pp. 3–44.]. Relatedness asymmetries due to haplodiploidy may then have modulated worker behavior secondarily, but already at the stage of eusociality [M. Andersson, *Annu. Rev. Ecol. Syst.* 15, 165 (1984)].