Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 5143–5147, May 1994
Evolution

DNA fingerprinting analysis of parent–offspring conflict in a bee

(*Augochlorella striata*/eusociality/haplodiploidy/sex ratio)

ULRICH G. MUELLER*, GEORGE C. EICKWORT†, AND CHARLES F. AQUADRO‡

*Section of Neurobiology and Behavior, Mudd Hall, †Department of Entomology, Comstock Hall, and ‡Section of Genetics and Development, Biotechnology Building, Cornell University, Ithaca, NY 14853

Communicated by C. D. Michener, February 22, 1994 (received for review December 20, 1993)

ABSTRACT Demonstrating the importance of haplodiploidy in the evolution of eusociality among the Hymenoptera (bees, wasps, and ants) requires estimation of four parameters: relatedness between cooperating individuals, effective mating frequency, sex ratio, and rates of worker reproduction. Multilocus DNA fingerprinting techniques permitted the precise determination of these parameters for the primitively eusocial bee *Augochlorella striata* (Halictidae). DNA fingerprints revealed an unprecedented resolution of genetic relationships within colonies, detecting factors such as intraspecific nest parasitism and diploid males that confounded estimates of relatedness and sex ratio, respectively. Parameter estimates (*i*) corroborate recent evidence for queen–worker conflict over the sex ratio and (*ii*) implicate the role of haplodiploidy in the evolution of worker behavior.

Augochlorella striata (Halictidae, Hymenoptera) is a primitively eusocial sweat bee with an annual colony cycle divided into a foundress phase and a worker phase (1–4). During the foundress phase (spring), foundresses construct a subterranean nest and provision a first brood (6 to 8 offspring) of both sexes. Upon emergence, first brood females remain in their natal nest as workers (worker phase, summer), while males leave and never return. Foundresses now cease all foraging, and their workers provision a second brood of male and female reproductives (8 to 13 sexuals). Reproductives mate in late summer, but only inseminated females overwinter to complete the colony cycle. In Ithaca, New York, virtually all nests are founded by single foundresses. In ≈40% of the nests, the foundress dies during the worker phase and is replaced by one of her first brood daughters (replacement queen). Such orphaned colonies are called "parasocial," in contrast to "eusocial" colonies that retain the foundress throughout the season (5). Queen supersedure alters genetic relatednesses in colonies. In parasocial colonies, workers are sisters of the replacement queen and rear nieces and nephews. In eusocial colonies, workers are daughters of the foundress and rear sisters and brothers.

Field experiments showed that eusocial colonies of *A. striata* produced a more female-biased sex ratio than parasocial colonies (3). Specifically, the average investment sex ratio (% male wet weight) was 32.8% for 12 eusocial colonies and 66.0% for 12 paired parasocial colonies. Because of haplodiploidy, workers in eusocial colonies are more closely related to female than to male reproductives (relatedness asymmetry present), while workers in parasocial colonies are equally related to female and male reproductives (relatedness asymmetry absent; refs. 5–8). Therefore, the observed sex-ratio difference suggested that workers facultatively adjusted the sex ratio and, in eusocial colonies, capitalized on the relatedness asymmetry by biasing the sex ratio toward the more closely related sisters (3, 5, 9, 10).

There exist two alternative explanations for the observed sex-ratio difference. First, sib-matings between replacement queens and their brothers may have produced diploid males in parasocial colonies. Under haplodiploidy, males develop from haploid eggs (unfertilized) and females from diploid eggs (fertilized), but diploid males can develop under specific mating types (11, 12). For bees, females develop from diploid eggs only if they are heterozygous at a single sex-determining locus. Diploid eggs develop into males if homozygous at the locus. Because of multiple alleles at this locus (11, 12), homozygosity occurs most frequently under inbreeding (e.g., sib-mating). Under sib-mating, therefore, half of the replacement queens produce broods with male-biased sex ratios (50% of their diploid brood develop into males).

As a second explanation, workers may have been more successful at laying male eggs in parasocial than in eusocial colonies. Higher rates of worker reproduction in parasocial colonies may be due to the lower "dominance potential" of replacement queens (they are about equal in size to their sister workers; foundresses are generally larger than their daughter workers; ref. 4). Unlike foundress queens, replacement queens therefore may be unable to prevent worker reproduction.

To quantify the extent of diploid males and worker reproduction, we generated multilocus DNA fingerprints (13) (Fig. 1) of all bees of the colonies used in the original study by Mueller (3). Fingerprints revealed that diploid males and worker reproduction contributed to but did not fully explain the sex-ratio difference between eusocial and parasocial colonies. Therefore, workers in *A. striata* appear to facultatively adjust the colony sex ratio depending on the presence or absence of a relatedness asymmetry.

MATERIALS AND METHODS

DNA Fingerprinting Methods. Standard protocols were used (14–16). Five to 12 μg of nuclear DNA was extracted in 2× CTAB buffer (15) from whole bees, digested with *Hae* III restriction enzyme, electrophoresed in a 0.8% agarose gel for 1700 V·hr at 30 V, Southern-blotted to a nylon filter, and hybridized overnight (*i*) at 60°C to radiolabeled 33.15 probe of Jeffreys *et al.* (13) and (*ii*) at 55°C to a radiolabeled species-specific probe (pUM-T17). The species-specific probe was a 1.4-kb *A. striata* insert excised from a recombinant phage in the vector Lambda-ZAP, isolated by using standard methods (17) to probe with a $(TTTA)_7$ oligonucleotide. Radiolabeled filters were washed [33.15: twice in 2× SSC (1× SSC = 0.30 M NaCl/0.03 M sodium citrate, pH 7) containing 0.1% SDS at room temperature, twice in the same buffer at 60°C, and once in 1× SSC at 65°C, each wash being for 15 min; pUM-T17: once in 2× SSC/0.1% SDS at room temperature and once in the same buffer at 55°C, each wash being for 15 min] and exposed without screens for 24–72 hr (33.15) or 10 days (pUM-T17).

DNA fingerprints were generated for all bees of 12 eusocial and 12 parasocial colonies (paired experimental colonies 1–5,

---

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

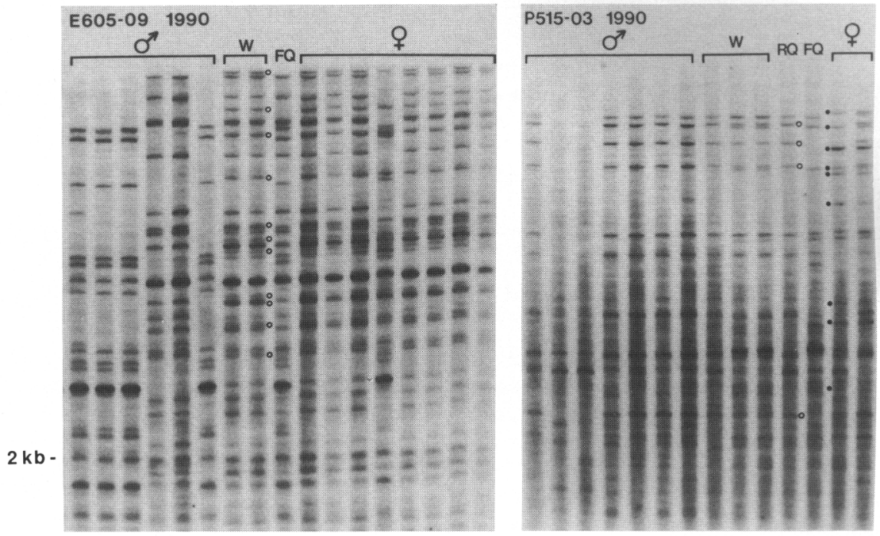

FIG. 1. DNA fingerprints of the queen, workers, and male and female reproductives of a eusocial *(Left)* and parasocial *(Right)* colony of *A. striata*, generated by hybridizing with the 33.15 probe (4). FQ, foundress queen; RQ, replacement queen; W, worker. The foundress was experimentally removed from the parasocial colony at the beginning of the worker phase, whereupon one of her daughters became the replacement queen. In the eusocial colony, workers are daughters of the foundress queen; in the parasocial colony, workers are sisters of the replacement queen. Open circles mark paternal bands (inherited from the foundress queen's mate) in first-brood females of both eusocial and parasocial colonies. Filled circles mark paternal bands (inherited from the replacement queen's mate) in second-brood females of the parasocial colony. In eusocial and parasocial colonies, all first-brood females shared the set of paternal bands. In the eusocial colony, first-brood females (workers) shared the set of paternal bands also with all second-brood females (supersisters). In the parasocial colony, paternal bands were identical across all second-brood females. Both foundress and replacement queens therefore mated only once. In the parasocial colony, paternal bands of second-brood females are not present in the foundress, indicating that the replacement queen had outbred and not mated with a brother.

7, 8, and 10–14 in ref. 3). Parasocial colonies included three generations (foundress, replacement queen and her sister workers, and putative offspring of the replacement queen); eusocial colonies included only two generations (foundress, workers, and second brood offspring). One male and one worker in two different parasocial colonies, and six female reproductives in one eusocial colony failed to provide fingerprints. Fingerprints of one eusocial colony were partly distorted and thus not scored but allowed determination of the mating frequency.

Scoring of DNA Fingerprints. Only bands larger than 2.0 kb were scored. Bands were scored as identical between two individuals if they showed congruent mobility and intensity (14). Band sharing proportions between two individuals (on the same gel) were calculated as twice the number of shared bands over the total number of bands in both individuals (14, 16); bands from both the 33.15 and pUM-T17 probings were used in the calculations.

Intraspecific Nest Parasitism. The frequency distribution of band-sharing proportions between foundresses and their putative female offspring showed extremely low values in six queen–worker dyads, suggesting that these workers were unrelated to the foundresses (see *Results*). Unrelated workers could have been offspring of conspecific nest parasites. To address this hypothesis, foundresses from nine nonexperimental colonies were genetically fingerprinted together with (*i*) their first broods (45 females, 19 males) reared from combs directly and (*ii*) 27 unrelated first-brood females selected at random from the same aggregation. This allowed generating band-sharing frequency distributions between (*i*) foundresses and their first-brood females reared from combs directly and (*ii*) foundresses and unrelated first-brood females from the same aggregation.

Mating Frequency and Relatedness. Effective mating frequency was determined for foundresses and replacement queens. The sets of paternal bands (present in a daughter but not in a queen) were identified in each daughter and compared between all daughters of the queen. Daughters sharing the same set of paternal bands had the same father. Therefore, the number of distinct sets of paternal bands among all daughters indicates the mating frequency of the queen. Relatednesses were calculated as described by Reeve *et al.* (18) as $r = (w - b)/(1 - b)$, where w is the average proportion of bands shared between *related* individuals and b is the average proportion of bands shared between *unrelated* individuals (all on the same gel). Relatednesses were calculated from bees on eight gels and then averaged. Reeve *et al.* recommend eliminating bands with frequencies >0.5 from the analysis, to minimize a downward estimation bias. This correction would eliminate paternal bands shared between full sisters (on gels with highly female-biased sex ratios) and thus was not followed, but this did not deflate relatednesses between full sisters because inclusion of paternal bands does not inflate relatednesses between unrelated individuals. Monomorphic bands were excluded from the analysis.

Sib-Mating and Diploid Males. Under sib-mating, paternal bands of a replacement queen's daughters are present also in the replacement queen's mother (foundress), as they were passed on from the foundress to one of her sons (replacement queen's mate) and then on to the replacement queen's diploid offspring. Under outbreeding, at least some paternal bands of the replacement queen's daughters are not present in the foundress. Comparing paternal bands of the replacement queen's daughters to the foundress therefore allows differentiation between sib-mating and outbreeding of replacement queens.

Worker Reproduction. Maternity of males was determined by matching fingerprints of males to putative mothers (queen and workers). Because males are produced parthenogenetically, a male's bands have to be present also in its mother. Worker reproduction therefore can be evident only if the fingerprint of a worker shows at least one band not present in the fingerprint of the queen; presence of this band in a male then identifies this male as a son of the worker. Consequently, worker reproduction of sons is more readily detected in eusocial colonies (≈50% of the bands, paternally inherited, differ between a worker and the queen) than in parasocial colonies (≈25% of the bands, maternally inherited, differ between a worker and the replacement queen, assuming they are full sisters; see *Results*). Fingerprints of males in parasocial colonies can be expected to match both the replacement queen and one of the workers if (*i*) the number of scored bands is low and (*ii*) bands show genetic linkage. Because bands in *A. striata* are linked (see *Results*), maternity could not be determined for some males in parasocial colonies: while some males could be matched only to the replacement queen (or only to a worker), others matched both the replacement queen and at least one worker.

A precise estimate of the extent of worker reproduction can be gained by determining the probability of detecting sons of workers. This probability can be calculated after (*i*) identifying sets of independently segregating bands to eliminate genetically linked bands (16) and (*ii*) eliminating non-informative bands (paternal bands and monomorphic bands shared between the replacement queen and her workers). Assuming that all workers were equally likely to produce sons and that fingerprinted workers represented a random subset of all workers present during the season (some workers died in the field), the probability of detecting sons of workers on the basis of a single, informative band "i" (present in at least one worker, but not in the replacement reproductive) can be calculated as $P_i = (0.5f)$, where f is the frequency of the band among all workers in a colony. The probability of detecting sons of workers on the basis of n independently segregating bands is $P_{\text{detect}} = (1 - g)$, where $g = ([1 - P_1]\cdot[1 - P_2]\cdot[1 - P_3]\cdot\,.\,.\,.\cdot[1 - P_n])$. The average, weighted probability of detection ($\overline{P}_{\text{detect}}$) for several colonies can then be calculated by weighing the P_{detect} of each colony by the colony's proportional contribution to the total number (T) of fingerprinted males in all colonies. If fingerprints identify x males as sons of workers, the expected proportion of sons of workers in the sample of T males is $x/[\overline{P}_{\text{detect}} \cdot T]$ (19).

RESULTS

Intraspecific Nest Parasitism. In five colonies, one ($n = 4$) or two ($n = 1$) workers were not daughters of the foundress (Fig. 2). In daily observations over several weeks prior to nest excavation (at least 15 hr of observation per nest), unrelated workers were behaviorally indistinguishable from daughter workers. Unrelated workers either (*i*) had joined colonies as adults in mid-season or (*ii*) were born in these colonies and were offspring of "egg-dumping" conspecifics that had parasitized nests during the foundress phase. DNA fingerprints of foundresses and their developing first brood (taken directly from combs of nine nests) revealed 4.4% (n = 45) unrelated females and no unrelated males (n = 19). Because all experimental colonies were initiated by single foundresses (3, 4), this indicates that at least some unrelated workers were offspring of "egg-dumping" conspecifics.

Mating Frequency and Relatedness. In each of the 24 fingerprinted colonies, all female offspring of a queen shared the same set of paternal bands and therefore represented one patriline (Fig. 1). Therefore, mating in *A. striata* was uniformly monandrous. Consequently, workers in eusocial colonies are expected to be (*i*) related to female reproductives by $r_{\text{female}} = 0.75$ and (*ii*) three times more related to female than to male reproductives ($r_{\text{male}} = 0.25$) (6–8). The expected r_{female} and r_{male} closely approximate the calculated relatednesses of 0.7856 (SE = 0.0112) and 0.2403 (SE = 0.0377), respectively, as described by Reeve *et al.* (18).

Sib-Mating and Diploid Males. Diploid males were found in one parasocial colony (Fig. 3). The replacement queen in that colony had mated with a brother, and six of her eight diploid offspring were diploid males. The six diploid males were significantly larger (average wet weight = 17.6 ± 1.6 mg) than males from outbred colonies (11.2 ± 2.5 mg; $t = 6.0$, df = 122, $P < 0.0001$), suggesting that diploid males develop on provisions "designated" for females (females in *A. striata* are 1.3 times larger than males; ref. 4). Replacement queens of all other parasocial colonies had outbred and did not produce diploid males (see Figs. 1 and 4).

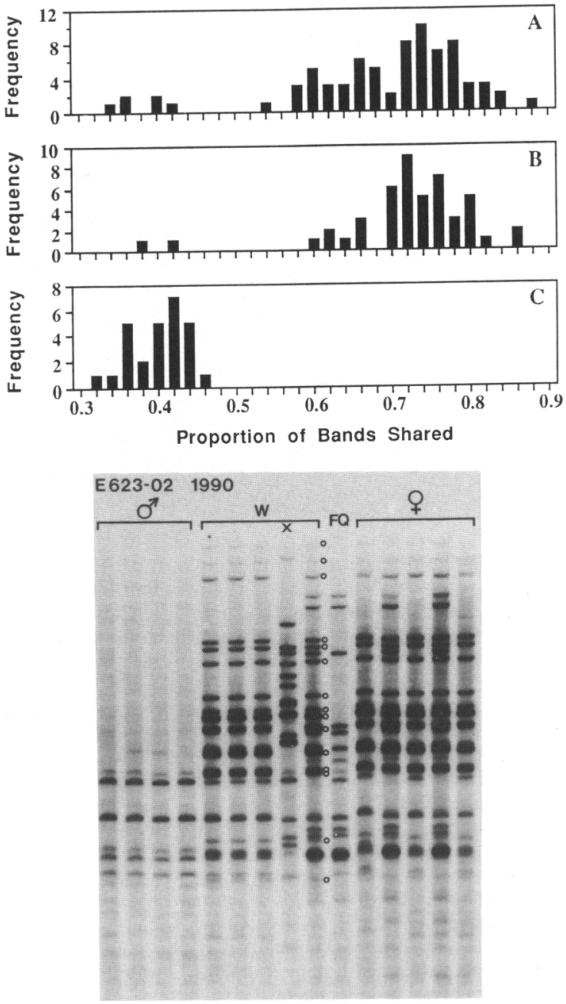

FIG. 2. (*Upper*) Frequency distributions of the proportions of bands shared between pairs of foundress queens and their workers from the 24 experimental colonies (76 pairwise comparisons) (*A*), foundress queens and their first-brood females reared from combs of nine nonexperimental colonies in the same population (45 pairwise comparisons) (*B*), and foundress queens of nine nonexperimental colonies and unrelated first-brood females from 17 nonexperimental colonies in the same population (27 pairwise comparisons) (*C*). The outliers at the low values of band-sharing proportions in *A* and *B* coincide with those of unrelated females in *C*. The frequency distributions therefore identify these outliers (4.4% of the first-brood females reared from combs and 7.9% of the workers) as unrelated to the foundress. Unrelated workers occurred in four eusocial colonies (11.6% of all workers) and once in a parasocial colony (3.0% of all workers). (*Lower*) Fingerprint of an entire colony with one unrelated worker (marked with an X) and four related workers. As in Fig. 1, open circles mark paternal bands of related workers.

Worker Reproduction. Worker reproduction was evident in parasocial (Fig. 4) but not in eusocial colonies. Four males (n = 74) in four different parasocial colonies were sons of workers; no such male (n = 49) occurred in eusocial colonies. In spite of the small number of unlinked informative bands in the fingerprints, banding patterns had considerable power to detect sons of workers (average probability of detection = 0.579). Thus, 9.3% (= 4/[0.579·74]) of the 74 males in

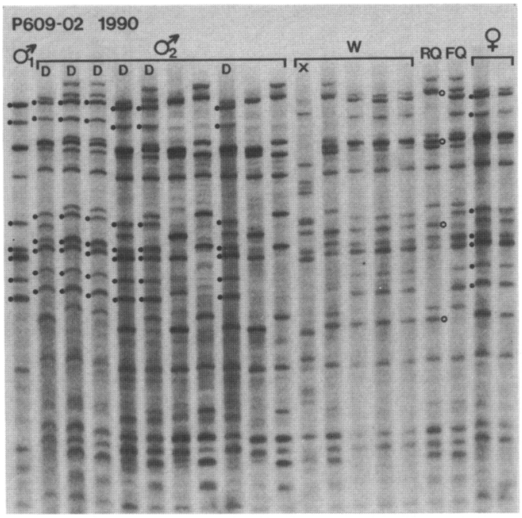

FIG. 3. Sib-mating and production of diploid males in a parasocial colony of *A. striata*. Filled circles mark the paternal bands present in the replacement queen's daughters and diploid sons (designated D). Under sib-mating, these paternal bands have to be present also in the replacement queen's mother (the foundress queen), as they were passed on from the foundress queen to one of her sons (the replacement queen's mate), and then on to the replacement queen's daughters and diploid sons. One first-brood male (shown far left) was caught in the field while leaving the colony and showed a fingerprint that matched this set of paternal bands. This male, or one of his brothers with an identical banding pattern, was the mate of the replacement queen. Counting the investment (wet weight) in diploid males as actual investment in females, this parasocial colony produced a sex ratio of 24.2% male investment. The particular parasocial colony was the only one showing a set of paternal bands that could be traced back to the foundress. All other parasocial colonies showed clear evidence of outbreeding (paternal bands not present in the foundress) (see parasocial colonies in Figs. 1 and 4). One worker (designated X) is not a daughter of the foundress.

parasocial colonies are predicted to be sons of workers. Daughters of workers were not found in eusocial or in parasocial colonies.

DISCUSSION

DNA fingerprints resolved the genetic architecture within colonies of *A. striata* with previously unattainable precision and permitted the (*i*) differentiation between related and unrelated bees to reveal intraspecific nest parasitism, (*ii*) estimation of mating frequency, (*iii*) measurement of relatedness, (*iv*) estimation of the extent of worker reproduction, and (*v*) detection of diploid males resulting from sib-mating.

Intraspecific Nest Parasitism. About 5% of first-brood females reared from combs directly and about 8% of the workers from active nests were offspring of "egg-dumping" conspecifics and thus were unrelated to their nestmates. Unrelated males were not found in the first brood. Nor was there evidence of egg-dumping in the second brood. DNA fingerprints provide the first unambiguous evidence of intraspecific nest parasitism in a social bee, strengthening anecdotal observations of cuckoo-like behavior (20, 21) in other halictids. This finding supports Brockmann's contention that intraspecific nest parasitism may be as common in bees as in other well-studied taxa (e.g., birds) (22).

For intraspecific parasitism to be adaptive, some offspring of parasitizing females have to become reproductives as (*i*) replacement queens, (*ii*) laying workers, or (*iii*) individuals that attempt independent nesting. (*i*) No parasocial nests in this study were headed by an unrelated replacement queen. However, given the low frequency of nest parasitism, evaluation of this avenue of reproduction for unrelated females requires a larger sample of parasocial colonies. (*ii*) Worker reproduction occurs in parasocial colonies at low rates (see below), but, possibly also because of small sample size, fingerprints did not reveal any offspring of unrelated workers. (*iii*) Both independent nest founding by first-brood females during the same season and the following year occurs at low rates in *A. striata* (4), but it is not known whether some of these independent founders are daughters of egg-dumping females.

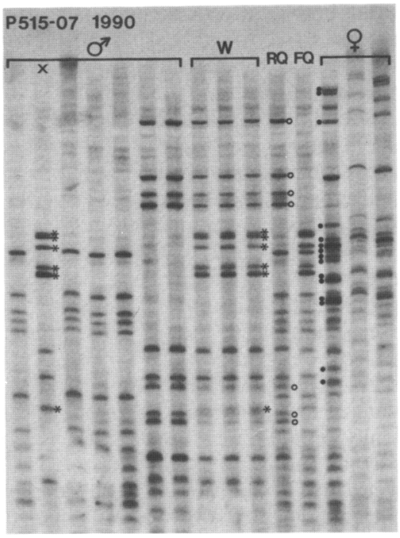

FIG. 4. Worker reproduction in a parasocial colony of *A. striata*. The male designated X shows bands (marked with asterisks) not present in the replacement queen but present in each of the workers; therefore, X was the son of a worker. Maternity of the two rightmost males is uncertain; they could be sons of either the replacement queen or one of her sister workers. Banding patterns of all other males can be matched only to the replacement queen, identifying her as the mother of these males. Only one informative, linked set of bands (marked with asterisks) is evident, with a frequency of 1 among all workers. Therefore, the probability of detecting sons of workers in this colony by using this fingerprint is 1/2.

Genetic distinctness of unrelated workers from the remaining workers in the same colony was not due to multiple mating by the queen. Under multiple mating, workers of all patrilines in a colony should show high band-sharing proportions with the queen (Fig. 2). However, the low band-sharing proportions of the six genetically distinct workers clearly identify them as unrelated. Therefore, unlike allozyme data (see, for example, ref. 21), DNA fingerprints allowed differentiation between two sources of genetic variation between workers: multiple maternity (intraspecific parasitism) and multiple paternity.

Mating Frequency and Relatedness. All 12 foundresses and all 12 replacement queens produced daughters of only one patriline. Therefore, eusocial colonies are characterized by extreme relatedness asymmetries and extreme queen–worker conflict over the sex ratio. Single mating in *A. striata* contrasts with previous reports that used behavioral and allozyme techniques to infer mating frequency in other halictid bees (21, 23, 24). All these studies suggested average mating frequencies of >1, but none could clearly reject single mating: allozyme data (21) are ambiguous because they fail to differentiate between multiple mating and multiple maternity (e.g., intraspecific nest parasitism), and observational data (23) are ambiguous because multiple copulations may not translate into multiple paternity of offspring. Therefore, single mating may be more common among halictid bees than has been previously inferred from behavioral and allozyme data.

Because of single mating, workers in eusocial colonies are expected to be 3 times more related to female ($r_{\text{female}} = 0.75$) than to male ($r_{\text{male}} = 0.25$) reproductives. The band-sharing procedure of Reeve *et al.* (18) for calculating relatednesses from fingerprints yielded estimates that closely matched these expected values (0.7856 ± 0.0112 and 0.2403 ± 0.0377, respectively), confirming the reliability of this method.

Worker Reproduction. Workers reproduce at low rates in parasocial colonies but apparently not in eusocial colonies. Worker reproduction therefore is not a dominant selective factor maintaining eusociality in *A. striata*. Other factors such as indirect reproduction or "hopeful reproduction" as replacement reproductives appear more important. The absence of worker reproduction in eusocial but not in parasocial colonies can be explained by several hypotheses. Mechanistically, egg-laying rates of the smaller replacement reproductives (4) may be limited, thus allowing for occasional worker reproduction. Second, the size advantage of foundresses may enable them to dominate workers, while replacement queens (of about equal size to their sister workers) may be unable to prevent worker reproduction. Third, theory (25) predicts that foundresses should be able to dictate a greater "reproductive skew" than replacement queens. These models assume that worker behavior is facultative and includes two strategies—helping a relative (accruing indirect fitness) and founding a nest independently. Because eusocial and parasocial colonies differ in the average worker–brood relatedness that affects indirect fitness (average worker–brood relatednesses in eusocial colonies is $r_{\text{male}} = 0.25$ and $r_{\text{female}} = 0.75$ and in parasocial colonies is $r_{\text{male}} = r_{\text{female}} = 0.375$), foundresses may be able to skew colony reproduction entirely in their own favor without losing the help of their workers. In contrast, replacement queens may have to yield some direct reproduction to workers that may otherwise attempt independent reproduction.

About 9% of the males in parasocial colonies were sons of workers. This value agrees with the estimate of 7–13% worker-produced sons reported previously (derived by inspecting ovarian development of workers) (3), suggesting that ovarian development is a reliable indicator of worker reproduction in parasocial colonies. This is surprising because it implies that (*i*) virtually all eggs laid by workers in parasocial colonies are male and are not removed by the queen, while (*ii*) workers in eusocial colonies either do not lay eggs (in spite of ovarian development that is comparable to that of workers in parasocial colonies; U.G.M., unpublished data) or have their eggs removed by the queen. Also surprising is the fact that workers produced only sons, even though about 40% of the workers were inseminated (4).

Diploid Males and Sex Determination. Packer and Owen (26) reported a single diploid male in an allozyme study of *A. striata* in Nova Scotia and estimated 2.6% diploid males in that population (assuming Hardy–Weinberg conditions). The present study confirms the existence of diploid males in this species (4.9% in the Ithaca population) but establishes that diploid males can result from inbreeding (sib-mating). Therefore, Hardy–Weinberg conditions may not hold for *A. striata*, and diploid males may occur more frequently than in outbred populations. The six diploid males occurred in a brood of eight diploid offspring (Fig. 4). Cook (12) calculated that the probability of six diploid males among eight diploid offspring is 0.145 under a single-locus model, but <0.0006 under a two-locus model. Thus, a single locus controls sex in *A. striata*, establishing this system of sex determination for bees also outside the well-studied family Apidae (12).

Sex-Ratio Biasing and Queen–Worker Conflict. Diploid males and worker reproduction confounded sex-ratio estimates of parasocial colonies. Because diploid males appear to develop on provisions "designated" for females (see above) and are probably sterile (12), they are in effect "females that die" during the time of parental investment. When counting diploid males as investment in females (Fig. 3), the average sex ratio of the 12 parasocial colonies (59.0% male investment) remains significantly more male-biased than the sex ratio of the 12 eusocial colonies (32.8%; $t = 2.83$, df = 11, one-tailed $P < 0.01$ after arcsine transformation). Similarly, when also correcting for 9.3% worker-produced sons in parasocial colonies (conservatively assuming that sons of workers replaced daughters of the replacement reproductive), sex ratios of parasocial colonies (49.7% male investment) remain significantly more male-biased ($t = 1.91$, df = 11, one-tailed $P < 0.05$). This eliminates both diploid males and worker reproduction as complete explanations, and relative relatedness asymmetries remain a viable explanation of the observed sex-ratio difference. Therefore, genetic factors intrinsic to the haplodiploid system of sex determination seem to play an important role in modulating worker behavior in *A. striata* (6–9).

We thank B. Danforth, S. Emlen, D. Pfennig, H. K. Reeve, T. Seeley, and P. Sherman for comments on the manuscript; M. Dean for oligonucleotides; M. Hamblin and D. Begun for help with cloning; and Z. Falin for rearing bees. The study was supported by a Grant-in-Aid-of-Research from Sigma Xi to U.G.M., a National Science Foundation Doctoral Dissertation Improvement grant to U.G.M. and G.C.E., a National Institutes of Health grant to C.F.A., and research funds from the Department of Entomology, Cornell University.

1. Ordway, E. (1965) *Insectes Soc.* **12**, 291–308.
2. Packer, L. (1991) *Behav. Ecol. Sociobiol.* **27**, 339–344.
3. Mueller, U. G. (1991) *Science* **254**, 442–444.
4. Mueller, U. G. (1993) Ph.D. thesis (Cornell Univ., Ithaca, NY).
5. Boomsma, J. J. (1991) *Trends Ecol. Evol.* **6**, 82–89.
6. Hamilton, W. D. (1964) *J. Theor. Biol.* **7**, 1–16.
7. Hamilton, W. D. (1972) *Annu. Rev. Ecol. Syst.* **3**, 193–232.
8. Trivers, R. & Hare, H. L. (1976) *Science* **191**, 249–263.
9. Seger, J. (1991) *Science* **254**, 384.
10. Mock, D. W. & Forbes, L. S. (1992) *Trends Ecol. Evol.* **7**, 409–413.
11. Bull, J. J. (1983) *Evolution of Sex Determining Mechanisms* (Benjamin Cummings, Menlo Park, CA).
12. Cook, J. M. (1993) *Heredity* **71**, 421–435.
13. Jeffreys, A. J., Wilson, V. & Thein, S. L. (1985) *Nature (London)* **314**, 67–73.
14. Westneat, D. (1990) *Behav. Ecol. Sociobiol.* **27**, 67–76.
15. Aquadro, C. F., Noon, W. A. & Begun, D. J. (1992) in *Molecular Genetic Analysis of Populations*, ed. Hoelzel, A. R. (Oxford Univ. Press, Oxford, U.K.), pp. 115–157.
16. Bruford, M. W., Hanotte, O., Brookfield, J. F. Y. & Burke, T. (1992) in *Molecular Genetic Analysis of Populations*, ed. Hoelzel, A. R. (Oxford Univ. Press, Oxford, U.K.), pp. 225–269.
17. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
18. Reeve, H. K., Westneat, D. F. & Queller, D. C. (1992) *Mol. Ecol.* **1**, 223–232.
19. Wrege, P. H. & Emlen, S. T. (1987) *Behav. Ecol. Sociobiol.* **20**, 153–160.
20. Packer, L. (1986) *J. N.Y. Entomol. Soc.* **94**, 458–466.
21. Kukuk, P. F. (1989) in *The Genetics of Social Evolution*, eds. Breed, M. D. & Page, R. E. (Westview, Boulder, CO), pp. 183–202.
22. Brockmann, H. J. (1993) *Trends Ecol. Evol.* **8**, 2–4.
23. Packer, L. (1986) *Can. J. Zool.* **64**, 2317–2324.
24. Page, R. E. (1986) *Annu. Rev. Entomol.* **31**, 297–320.
25. Reeve, H. K. & Ratnieks, F. A. (1993) in *Queen Number and Sociality in Insects*, eds. Keller, L. & Cherix, D. (Oxford Univ. Press, Oxford, U.K.), pp. 45–85.
26. Packer, L. & Owen, R. E. (1990) *Heredity* **65**, 241–248.