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Author(s): Robert A. Krebs and Thersese A. Markow

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COURTSHIP BEHAVIOR AND CONTROL OF REPRODUCTIVE ISOLATION IN *DROSOPHILA MOJAVENSIS*

ROBERT A. KREBS<sup>1</sup> AND THERESE A. MARKOW

*Department of Zoology, Arizona State University, Tempe, AZ 85287*

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One of the greatest problems in understanding speciation stems from the fact that the process cannot be observed in its entirety. One is usually limited either to making inferences from species that have already formed or to investigating differences between "incipient" species, which, besides being relatively rare, may have various features rendering them unsuitable candidates for empirical studies. However, when they can be identified, populations that appear to be evolving reproductive isolation at either the pre- or postcopulatory level provide rare opportunities to study the conditions under which isolation evolves as well as the actual physical and behavioral nature of the isolation itself.

The *repleta* group of *Drosophila* has large numbers of closely related species (Throckmorton, 1975; Wasserman, 1982) for which the elements controlling genetic isolation can be elucidated (e.g., see Zouros, 1986a, 1986b). One *repleta* species in particular, *D. mojavensis*, breeds in necrotic cacti in several different regions of the deserts of North America and has been the subject of extensive studies of genetic and ecological differentiation (see review by Heed [1982]). *Drosophila mojavensis* races vary with respect to a number of ecological factors that could influence their differentiation, the most obvious being a shift in host plants. In the Sonora region, the major host plant is organ-pipe cactus (*Stenocereus thurberi*), although other columnar cacti are occasionally used, including cina cactus (*S. alamosensis*), which is the major host plant of the sibling species, *D. arizonensis*. On the Baja California peninsula, the major host plant of *D. mojavensis* is agria cactus (*S. gummosus*).

Reproductive isolation has been observed in the laboratory between strains of *D. mojavensis* derived from the Sonora region and strains derived from Baja California (Wasserman and Koepfer, 1977; Zouros and d'Entremont, 1980). Various explanations have been proposed to underlie the isolation, including character displacement (Zouros and d'Entremont, 1980) and body-size differences between the races (Brazner, 1983). Though the responsible factors remain obscure, a genetic basis for this sexual isolation is apparent from the selection experiments of Koepfer (1987a). If the nature of the isolating mechanisms could be elucidated, the qualitative and quantitative features of those characters that reduce certain types of matings could then be determined. One clue comes from the observation that the significant reproductive isolation between *D. mojavensis* populations is largely attributable to a re-

duction in matings between Baja-region males and Sonora-region females. Furthermore, male and female reproductive behaviors do not contribute equally to the observed isolation (Koepfer, 1987b).

In the present study, experiments were designed to identify the specific point or points during the entire period of precopulatory male-female interactions at which interpopulation pairings are impeded. We have done this by directly examining the courtship progress of single pairs of flies and, in addition, by employing the more traditional "choice" experiments to assess precourtship discrimination by males.

MATERIALS AND METHODS

*Strains of Drosophila mojavensis*.—The strains employed in this study (and the collection sites and years) were as follows: S1A and S1B (Santa Rosa mountains, AZ; 1986 and 1980, respectively), S2 (Desemboque region, Sonora, Mexico; 1986), S3 (Topolobampo, Sinaloa, Mexico; 1974), B1 (San Lucas, Baja California, Mexico; 1986), B2 (Punta Prieta, Baja California, Mexico; 1985), B3 (La Paz, Baja California, Mexico; 1974), and B4 (Ensenada, Baja California, Mexico; 1980). All strains were initiated with a minimum of 80 flies either reared from necrotic cactus or aspirated from their host plants, and the strains were maintained in mass cultures of at least eight bottles. Flies from the Baja California peninsula were reared from agria cactus, and those from the mainland Sonoran Desert were reared from organ-pipe cactus, with the exception of the Desemboque strain [S2], which was collected and reared from agria, which is present in this one area of mainland Mexico. Those strains not collected by the authors were provided by W. Heed at the University of Arizona or J. Fogleman at the University of Denver. Strains S1A, S1B, S2, and S3 are all considered to belong to the Sonora race (geographic region); strains B1–B4 all belong to the Baja race. Henceforth, those are referred to as Sonora and Baja flies, respectively.

*Culture Procedures*.—All flies were reared in half-pint bottles containing standard cornmeal-molasses-yeast medium with buffered propionic acid and seeded with live yeast. Virgin males and females were separated under light ether anesthesia 1–8 hours after eclosion and stored in 8-dram vials with live yeast, eight flies/vial, until used in experiments. Males were ten days of age and females were six days of age when utilized in experiments, unless stated otherwise. Flies were maintained in an environmental chamber on a 13:11 day: night cycle with a day temperature of 26–27°C and a night temperature of 20–21°C.

*Behavioral Discrimination Prior to Courtship*.—The possibility that geographic populations of *D. mojavensis*

<sup>1</sup> Present address: Department of Animal Science, University of New England, Armidale, NSW 2351, Australia.

sis are differentially stimulated to court each other was examined in two sets of experiments. In the first, two males, one from each geographic area (distinguished by small wing clips), were placed in an 8-dram vial with a single female from one of the same strains as the males. The type of the first male to begin courting was recorded. In the second set of experiments, two females, one from each geographic area (distinguished by small wing clips), were placed with one male from the same strain as one of the females. The type of the first female to be courted was recorded. In all experiments, the single fly (male or female) was the last to be introduced, and the clipping was alternated between replications. In these experiments, both males and females were 10 days old. The two sets of experiments were conducted in different years and employed different strains: 1982 (S1B, S3, B3, and B4) and 1987 (S1A and B1).

*Timing of Courtship Breakdown.*—Pairs of flies were aspirated into 8-dram food vials and observed for 20 minutes. The “landmarks” to which courtship progressed were scored as in Markow (1981). Courtship was scored when a male vibrated his wings at a female or licked a female. Females indicate their receptivity to a male by a characteristic spreading of the wings. Males respond by an immediate copulation attempt. The time until the male courted (courtship latency), the time from courtship initiation until female acceptance (receptivity), and the total time until a successful mating was observed (copulation latency) were scored. The proportions of flies reaching these courtship landmarks were evaluated by *G*-statistic frequency analyses of  $2 \times 2$  tables, and the actual times required to reach each landmark were analyzed both by GLM procedures with Duncan’s multiple-range test and by Wilcoxon rank sums (SAS Institute, 1985) with a nonparametric multiple-range test (Zar, 1984 pp. 199–201). Parametric and nonparametric tests were in close agreement.

In order to evaluate the role of geographic isolation, we conducted two kinds of control pairings, within strain and within geographic race, along with the intergeographic pairings. Because control pairings were always run simultaneously with experimental pairings, final sample sizes for control groups were larger. One hundred or more presentations were made for each comparison.

*Measurements of Body Size.*—Sonora flies are, on average, larger than Baja flies, and this size difference may be a factor in the asymmetry in mating success. Therefore, all flies were measured for thorax length using an ocular micrometer. The importance of size for courtship and mating behaviors was analyzed by two methods: by seeking correlations between size and the times required to reach the behavioral landmarks and by comparing sizes of successful and unsuccessful flies. Correlations of male and female size with courtship latency, receptivity, and copulation latency were calculated for experimental and control groupings. Because 144 correlations with size were run (two sexes, three behaviors, six between-strain comparisons, and four presentation groups within each between-strain comparison), seven “significant” correlations were expected due to chance alone.

*Analysis of Courtship-Bout Lengths and Male Vigor.*—Courtship-bout lengths were examined by videotaping courtships of individual pairs of flies (Markow

and Hanson, 1981; Markow, 1987). Flies to be videotaped were reared identically to those above, except that temperature was held constant at 24°C. Flies were aspirated into a round mating chamber (depth 6 mm, top diameter 18 mm, and bottom diameter 7.5 mm) and videotaped with a JVC color video camera equipped with a 1:2 macrozoom lens.

Flies from strains S1A, S2, B1, and B2 were paired in a completely randomized design. Each pair was filmed for ten minutes. Videotapes were then replayed to allow measurement of all courtship bouts, interbout lengths, and total courtship time until matings occurred. Male vigor, a modified courtship index (Tompkins et al., 1980), was defined as the percentage of time spent courting from first courtship until the end of filming or observed copulation (Gromko and Newport, 1988).

## RESULTS

*Behavioral Discrimination Prior to Courtship.*—In tests for the first female to be courted (Table 1A), males did not appear to discriminate between females of different geographic origin (combined data  $X^2 = 0.3$ , *d.f.* = 1,  $P > 0.5$ ). Testing for female-dependent differences in the first male to begin courtship (Table 1B) demonstrated that Sonora males typically court before Baja males (combined data  $X^2 = 35.0$ , *d.f.* = 1,  $P < 0.001$ ). Tests in which Sonora males courted first occurred twice as often as tests in which Baja males courted first (Table 1B). This comparison between males of the two races gave similar results regardless of female race used in the tests (heterogeneity  $X^2 = 0.99$ , *d.f.* = 1,  $P > 0.3$ ).

*Timing of Courtship Breakdown.*—There was no significant heterogeneity with respect to the time periods of the experimental replicates or within geographic locality. Data for strains within region were thus pooled. Figure 1 shows the proportions of flies from each group that reach the various behavioral landmarks. As would be predicted from the first experiments, Sonora males courted all females more frequently than did Baja males. Baja males, however, were significantly more likely to court their own females (81% of 820 trials) than they were to court Sonora females (76% of 403 trials;  $G = 4.21$ , *d.f.* = 1,  $P < 0.05$ ). The greatest difference between pairings was in female receptivity. Sonora females paired with Baja males have a significantly lower acceptance frequency (73% of 306 courting pairs) than when they were paired with Sonora males (83% of 746 courting pairs;  $G = 12.5$ , *d.f.* = 1,  $P < 0.001$ ). Baja females accepted both Sonora (92% of 371) and Baja (90% of 662) males with equal frequency ( $G = 0.18$ , *d.f.* = 1, ns).

An additional decline in mating success was associated with a failure of some males to copulate successfully after being given the acceptance signal when they were paired with females of the alternate geographic origin. The frequency of failure after females had given the acceptance signal differed among the four experimental combinations (Table 2;  $G = 15.87$ , *d.f.* = 3,  $P < 0.01$ ) and was highest when Baja males were presented to Sonora females. Most presentations ended in a mating within the 20-minute observation period once a female acceptance signal was observed. However, the mating frequency among these males also differed (Table 3: number accepted minus number mated;  $G = 12.74$ , *d.f.* = 3,  $P < 0.01$ ). Again this significant difference was the effect of the greater failure rate of

TABLE 1. A) Courtship initiation in *D. mojavensis* males with a choice of Baja and Sonora females; B) first male to court (Baja or Sonora) when pairs of males were tested with single females.

| A.           |                | First female courted |        |    |
|--------------|----------------|----------------------|--------|----|
| Male strains | Female strains | Baja                 | Sonora | P  |
| B1           | B1, S1A        | 23                   | 23     | ns |
| B3           | B3, S3         | 46                   | 52     | ns |
| B4           | B4, S3         | 11                   | 9      | ns |
|              |                | 80                   | 84     | ns |
| S1A          | B1, S1A        | 24                   | 26     | ns |
| S3           | B3, S3         | 42                   | 45     | ns |
| S3           | B4, S3         | 10                   | 12     | ns |
|              |                | 76                   | 83     | ns |
| Combined:    |                | 156                  | 167    | ns |

| B.           |                | First male to court |        |       |
|--------------|----------------|---------------------|--------|-------|
| Male strains | Female strains | Baja                | Sonora | P     |
| B3, S1B      | B3             | 25                  | 43     | <0.05 |
| B3, S3       | B3             | 25                  | 43     | <0.05 |
| B4, S3       | B4             | 24                  | 37     | ns    |
|              |                | 74                  | 123    | <0.01 |
| B3, S1B      | S1B            | 16                  | 30     | ns    |
| B3, S3       | S3             | 28                  | 60     | <0.05 |
| B4, S3       | S3             | 25                  | 51     | <0.05 |
|              |                | 69                  | 141    | <0.01 |
| Combined:    |                | 148                 | 264    | <0.01 |

Baja males to copulate with Sonora females after they had been accepted (14 of 214 cases).

The average times required to reach the various behavioral landmarks for each type of pairing are shown in Table 3. Obviously, these times could only be computed for individuals reaching the landmark. Therefore, the sum of courtship latency and receptivity does not necessarily equal copulation latency. Sonora males courted significantly earlier than Baja males, regardless of female strain (Table 4A). Conversely, Baja females had significantly shorter receptivity times than Sonora females (Table 4B). In general, Sonora males were faster to initiate courtship, and Baja females were faster to accept males. As a result, overall copulation latencies within races were identical, while pairings involving Sonora males and Baja females had the shortest copulation latencies. As would be expected, Baja males paired with Sonora females were associated with the slowest copulation latencies (Duncan's multiple-range test,  $P < 0.05$ ).

*Analysis of Courtship-Bout Lengths and Male Vigor.*—Courtship-bout lengths, interbout lengths, and courtship vigors, derived from videotaped pairings, are reported in Table 5. The only significant differences among races occurred for bout lengths ( $F_{[3, 139]} = 9.26$ ,  $P = 0.01$ ); courtships of Sonora females (by either type of male) were longer than courtships of Baja females. However, this difference was clearly caused by short acceptance latencies of Baja females in general. Courtship vigor did not differ significantly among combinations of pairings between the races, regardless of

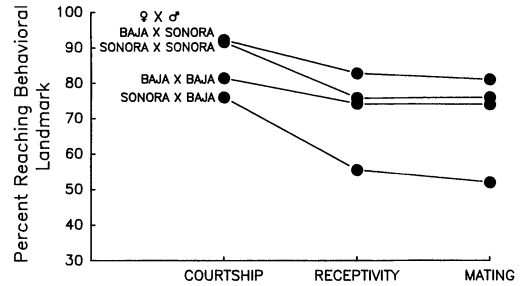


FIG. 1. Frequencies for single pairs of *Drosophila mojavensis* reaching the behavioral landmarks of courtship, acceptance of males (receptivity), and mating. Sonora and Baja strains were pooled for comparison of geographic origin. Sample sizes were 800 pairings within each geographic region (Sonora × Sonora and Baja × Baja) and 400 pairings between regions (Baja × Sonora and Sonora × Baja).

whether actual or arcsine-transformed values were analyzed.

*Body Size and Courtship Behavior.*—Neither male nor female body size was consistently correlated with any courtship parameter. Ten “significant” ( $P < 0.05$ ) correlations were found (144 correlations run), but there was no pattern with respect to either the sign of the correlation or the particular behaviors (courtship latency, receptivity, and copulation latency). Half of the significant correlations were found for only one of the three replicates performed for control groups. For example, correlation coefficients for male size and copulation latency calculated for the three replicates of S2 control pairings gave values of  $-0.25$  ( $N = 91$ ,  $P < 0.05$ ),  $0.14$  ( $N = 92$ , ns) and  $-0.10$  ( $N = 69$ , ns).

Similarly, comparing mated and nonmated individuals did not suggest any size effect on mating success of males in this species. The difference in mated ( $0.9358 \pm 0.0012$  [SE] mm;  $N = 1,657$ ) and nonmated ( $0.9337 \pm 0.0021$  mm;  $N = 623$ ) male thorax lengths was only  $0.0021$  mm ( $t$  test,  $t = 0.90$ , ns). Unmated females ( $1.0025 \pm 0.0024$  mm;  $N = 626$ ) were slightly larger than mated ones ( $0.9953 \pm 0.0015$  mm;  $N = 1,670$ ) ( $t = 2.52$ ,  $P < 0.01$ ), but this effect was consistent both within and among strains, which suggests that there is a size effect on female mating propensity and not that larger females differed from small females in discriminating between Sonora and Baja males.

DISCUSSION

In seeking the behavioral factors associated with the reduction in matings between Baja males and Sonora females, we have characterized the qualitative and

TABLE 2. Failure to copulate after first female acceptance for pairings within and between the Baja and Sonora strains of *D. mojavensis*.

| Pairing |        | Number succeeded | Number failed | Percentage failure (SE) |
|---------|--------|------------------|---------------|-------------------------|
| Female  | Male   |                  |               |                         |
| Baja    | Sonora | 276              | 57            | 0.17 (0.04)             |
| Sonora  | Sonora | 520              | 99            | 0.16 (0.02)             |
| Baja    | Baja   | 481              | 120           | 0.20 (0.03)             |
| Sonora  | Baja   | 161              | 63            | 0.28 (0.04)             |

TABLE 3. Latencies (in seconds) for single pairs of *Drosophila mojavensis* reaching the behavioral landmarks of courtship, acceptance of males (receptivity), and mating. The superscript letters denote groups that differ significantly, based on Duncan's multiple-range test ( $P < 0.05$ ).

| Pairing (♀ × ♂) | Latency ± SE (N)            |                            |                             |
|-----------------|-----------------------------|----------------------------|-----------------------------|
|                 | Courtship                   | Receptivity                | Mating                      |
| Baja × Sonora   | 162 ± 9 (371) <sup>a</sup>  | 54 ± 6 (333) <sup>a</sup>  | 218 ± 12 (325) <sup>a</sup> |
| Sonora × Sonora | 181 ± 7 (746) <sup>a</sup>  | 75 ± 6 (619) <sup>b</sup>  | 252 ± 9 (610) <sup>a</sup>  |
| Baja × Baja     | 214 ± 8 (662) <sup>b</sup>  | 40 ± 5 (601) <sup>a</sup>  | 253 ± 9 (588) <sup>a</sup>  |
| Sonora × Baja   | 215 ± 12 (306) <sup>b</sup> | 87 ± 12 (224) <sup>b</sup> | 299 ± 18 (210) <sup>b</sup> |

quantitative components of courtship of *D. mojavensis*. We have asked whether these parameters differed in those pairings which, by their failure to result in copulations, were responsible for the significant degree of sexual isolation observed between these populations in the laboratory.

The results indicate that the reduction in matings was due to factors operating at three levels of male-female interaction: before courtship initiation, during courtship, and at the time copulation was attempted. Several of the experiments reported above had the potential to reveal differential precourtship attractiveness between flies from different geographic strains. When Baja males were simultaneously presented with Baja and Sonora females, the females were equally likely to be approached and courted first. Among the single pairs, those Baja males that courted displayed equally slow courtship latencies whether paired with a Sonora or a Baja female. While these data do not show discrimination at the precourtship level, the results of the courtship-landmark experiment, in which Sonora females were significantly less likely to be courted by Baja males than were Baja females, indicates that some precourtship mechanisms may be operating. We know from earlier studies (Markow, 1981, 1982) that *D. mojavensis* males from Sonora strains possess the ability to discriminate between *D. mojavensis* and *D. arizonensis* females prior to courtship initiation and to discriminate among females of their own strain on the basis of female mating history and female size. Precourtship discrimination by males is clearly assuming a role in the evolution of behavioral isolation in *D. mojavensis*.

Because Baja males are slower to initiate courtship,

they are at a disadvantage in tests for isolation that employ a choice or competition design (Zouros and d'Entremont, 1980; Koepfer, 1987a, 1987b). However, the reduced success of Baja males with Sonora females in the case of single pairs observed here cannot be due solely to their being outcompeted by more vigorous males. The proportion of single pairs in which Sonora females are receptive to courtship by Baja males is greatly reduced compared to other combinations, suggesting a lack of fit between the courtship stimuli provided by Baja males and the receptivity requirements of Sonora females.

In addressing the nature of the rejection of courting Baja males by Sonora females, we examined the possibility that, after courtship had begun, the males were not courting vigorously enough or in long enough bouts to stimulate receptivity. This hypothesis was not supported by our results, as the courtship-bout lengths, interbout intervals, and male courtship vigor were found to be similar in experimental and control pairings. While bout lengths were longer when Sonora females were used, no between-strain difference in bout lengths based upon the type of male doing the courting were detected. The failure of Baja males to induce receptivity in Sonora females must be due to other aspects of the information being exchanged. Even when Sonora females were willing to accept Baja males, some males were unsuccessful in achieving intromission. This observation is consistent with an hypothesis of morphological change in the genitalia between Baja and Sonora *D. mojavensis* (A. Ruiz, W. B. Heed, and M. Wasserman, unpubl.).

The behavioral elements responsible for divergence

TABLE 4. Two-way general linear-model analyses of A) courtship latency ( $R^2 = 0.011$ ) and B) female acceptance of males (receptivity;  $R^2 = 0.017$ ) for pooled comparisons between Sonora and Baja populations of *Drosophila mojavensis*.

| Source                | d.f.  | Type-III SS | F     | P      |
|-----------------------|-------|-------------|-------|--------|
| A. Courtship latency: |       |             |       |        |
| Male                  | 1     | 847,818     | 21.07 | 0.0001 |
| Female                | 1     | 40,905      | 1.02  | 0.3134 |
| Male × female         | 1     | 38,254      | 0.95  | 0.3296 |
| Error                 | 2,081 | 84,633,475  |       |        |
| B. Receptivity:       |       |             |       |        |
| Male                  | 1     | 3,220       | 0.02  | 0.8948 |
| Female                | 1     | 433,401     | 23.55 | 0.0001 |
| Male × female         | 1     | 61,895      | 3.36  | 0.0669 |
| Error                 | 1,776 | 32,684,509  |       |        |

TABLE 5. Mean  $\pm$  SE courtship-bout lengths, interbout-intervals, and courtship vigor (as a proportion of time spent courting following the initiation of the first courtship). Numbers in parentheses are sample sizes.

| Pairing ( $\sigma \times \delta$ ) | Courtship-bout length (sec) | Interbout interval (sec) | Courtship vigor      |
|------------------------------------|-----------------------------|--------------------------|----------------------|
| Baja $\times$ Sonora               | 15.7 $\pm$ 1.7 (37)         | 35.6 $\pm$ 6.4 (24)      | 0.73 $\pm$ 0.10 (39) |
| Sonora $\times$ Sonora             | 22.0 $\pm$ 3.8 (36)         | 22.8 $\pm$ 6.2 (24)      | 0.88 $\pm$ 0.08 (37) |
| Baja $\times$ Baja                 | 17.5 $\pm$ 1.7 (35)         | 26.2 $\pm$ 8.0 (23)      | 0.87 $\pm$ 0.09 (38) |
| Sonora $\times$ Baja               | 22.7 $\pm$ 2.4 (35)         | 21.7 $\pm$ 3.5 (28)      | 0.74 $\pm$ 0.12 (38) |

in precourtship mate recognition do not appear to be detectable by visual inspection of courtship between *D. mojavensis* males and females. The possibility that male courtship song differs between Baja and Sonora populations was investigated by Ewing and Miyan (1986), but no significant differences were detected. The only other potential factors are tactile or chemosensory. Observation of courting pairs of any species in the *mulleri* complex of *Drosophila* reveals two striking features. First, species do not differ in their overall courtship structure, allowing for little exchange of visual information. Second, during most of the time that a male is courting, his proboscis and foretarsi are in contact with the female. Because the proboscis, foretarsi, and ovipositor bear numerous chemoreceptors (Dethier, 1962), it is likely that chemical information is being exchanged during (as well as prior to) courtship and that the population differences in courtship success may be mediated by this sensory mode. A preliminary analysis of the cuticular lipids of *D. mojavensis* indicates that surface hydrocarbons are sexually dimorphic (E. Toolson, R. Howard, L. Jackson, and T. A. Markow, unpubl.).

The model proposed by Kaneshiro (1980) to explain the origin of asymmetrical behavioral isolation as a result of founder events does not apply to *D. mojavensis* (Giddings and Templeton, 1983). An alternative hypothesis for the evolutionary origin of the differences in male and female mate-recognition characters was proposed by Wasserman and Koepfer (1977) and Zouros and d'Entremont (1980). They argue that selection on the mate-recognition system of *D. mojavensis* to avoid mating with *D. arizonensis* in areas of sympatry has led to increased discrimination by *D. mojavensis* females from Sonora. Our observations on sexual isolation among several strains, the increased receptivity time of Sonora females, and the increased likelihood of Baja males to fail to copulate successfully after an acceptance signal by Sonora females is consistent with their hypothesis.

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### COMPARATIVE ANALYSIS OF SEX-INVESTMENT RATIOS IN SLAVE-MAKING ANTS

ANDREW F. G. BOURKE

*Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, U.K.*

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Trivers and Hare (1976) combined sex-ratio theory (Fisher, 1930) and kin-selection theory (Hamilton, 1964a, 1964b) to produce a genetic-relatedness hypothesis of sex-ratio determination in social insects. The hypothesis predicts that the allocation of sex investment should be determined by the relatedness between the party controlling investment and the females and males receiving investment. In nonparasitic ants, the workers are thought to control investment, so that, if there is one maternal queen per colony (monogyny) and if queens mate singly, randomly breeding species should produce a 3:1 female:male investment ratio. But in monogynous slave-making ants, worker control of investment is unlikely, because all brood is raised by captive, nonconspecific "slaves." Therefore, in these species, queens should achieve their preferred 1:1 investment ratio (Trivers and Hare, 1976). However, if mating is nonrandom, such that related males compete for females (local mate competition [Hamilton, 1967]), as can occur especially in inbreeding populations, ant sex ratios should be female-biased, regardless of who raises brood (Alexander and Sherman, 1977). Thus, slave-makers permit a critical test between the genetic-relatedness and local-mate-competition sex-ratio hypotheses (Nonacs, 1986).

For this reason, Bourke et al. (1988) recently investigated sex investment, relatedness, and mating structure in a Swedish population of the monogynous slave-making ant *Harpagoxenus sublaevis*. They found that queens mate singly and that, although all queens in Swedish *H. sublaevis* populations are permanently wingless (Buschinger and Winter, 1975), inbreeding was absent. Furthermore, the wide dispersal of the winged males suggested that local mate competition without inbreeding did not occur. *H. sublaevis* workers possess ovaries and compete to lay haploid (male) eggs (Bourke, 1988a). Although the production of males by

workers also influences sex investment under the genetic-relatedness hypothesis (see below), observed levels were insufficient to perturb the theoretical ratio appreciably. The mean per-colony proportion of dry weight investment in *H. sublaevis* queens was 0.540 ( $N = 37$  colonies), which was not significantly different from 0.5 (1:1 investment). Hence the results of Bourke et al. (1988) supported the genetic-relatedness hypothesis.

In this paper, I evaluate previously published data on sex ratios in four obligate and one "degenerate" (see later) slave-making ant species and compare them with the Swedish *H. sublaevis* data of Bourke et al. (1988) to provide a comparative test of sex-ratio hypotheses in slave-makers. The relevant data come from the studies of Wesson (1939) on *Harpagoxenus americanus*, Talbot (1957) on *Leptothorax duloticus*, Buschinger et al. (1975) on a West German *H. sublaevis* population, and Winter and Buschinger (1983) on *Epimyrma ravouxi* and *E. krausseii* (the "degenerate" slave-maker). Three of these five case studies (all except those of Winter and Buschinger [1983]) have been considered in other papers analyzing slave-maker sex ratios (Trivers and Hare, 1976; Alexander and Sherman, 1977; Nonacs, 1986). In this paper I bring together all relevant slave-maker sex-ratio data gathered to date and critically discuss problems with some of the data which the previous analyses did not consider. I also quantify for the first time the impact of the production of males by workers on slave-maker sex ratios.

Bourke et al. (1988) found that, in *H. sublaevis*, most worker-produced males (up to 22% of all males) came from orphaned workers (i.e., workers released from queen inhibition of ovarian development by the queen's death). The expected effects of male production by queenless workers on the sex ratio were modelled by Taylor (1981), who assumed queen control of investment (as hypothesized for slave-makers) and by Non-