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Aggregation and Mate Location in the Red Milkweed Beetle (Coleoptera: Cerambycidae)

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We examined behavioral mechanisms underlying aggregation and mate location in the red milkweed beetle, Tetraopes tetrophthalmus *(Forster) (Coleoptera: Cerambycidae). Larvae of this species feed on rhizomes of common milkweed,* Asclepias syriaca *L., and adults feed on the flowers and foliage, aggregating on individual stems within milkweed patches. Adults preferred to aggregate on milkweeds that had multiple, large inflorescences. Males actively searched for females, often flying between host plants. Mate location did not appear to involve long-range pheromones or vision, but rather males landed on milkweed stems arbitrarily, whether or not females were present. Males remained for longer periods, and so tended to accumulate, on milkweed stems that had female-biased sex ratios. We conclude that aggregation of* T. tetrophthalmus *is cued by host plant characteristics but dynamically influenced by the sex ratio of conspecifics present on individual stems.*

KEY WORDS: *Tetraopes; Asclepias syriaca*; dispersal; mating; host plant quality; pheromone.

INTRODUCTION

Locating a mate is critical for sexually-reproducing organisms, thus there is selection for behaviors that improve the probability of encountering a mate (Price, 1997; Wells *et al.*, 1998). For example, male insects may be drawn to areas where females are concentrated, such as where they emerge, forage, or

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oviposit (Thornhill and Alcock, 1983). Aggregation provides an opportunity for individuals to assess the quality of potential mates and improves detection of predators and defense against them (Thornhill and Alcock, 1983; Godfray and Cook, 1997). Aggregation may be cued by visual signals (synchronous flashing of some firefly species), sound (stridulating Orthoptera), or olfaction (bark beetles [Thornhill and Alcock, 1983]). Females and males also may be brought together by a mutual attraction to an environmental stimulus, such as volatile compounds emanating from larval hosts of woodboring beetles (Hanks, 1999). For many species, however, the behavioral mechanisms underlying aggregation are not known.

We examined aggregation behavior and mate location of the red milkweed beetle, *Tetraopes tetrophthalmus* (Forster) (Coleoptera: Cerambycidae). Adults of this univoltine species feed on leaves and flowers of the common milkweed, *Asclepias syriaca* L. (Chemsak, 1963). This perennial herb has multiple, leafy, simple stems, 1–2 m tall, and umbels produced in the leaf axils (Gleason and Cronquist, 1963). Common milkweed occurs in patches of a few to thousands of stems (Hartman, 1977; McCauley *et al.*, 1981). Adult *T. tetrophthalmus* mate on milkweeds, and females deposit clutches of 5–20 eggs in the stems of grasses near milkweed plants or in dead milkweed stems of the previous year (Gardiner, 1961; Hartman, 1977; McCauley, 1983). Eggs hatch after about 2 weeks and neonates drop to the ground and burrow in the soil to feed on milkweed rhizomes (Gardiner, 1961; McCauley and Reilly, 1984). The larvae overwrinter as prepupae in earthen cells (Hartman, 1977). In central Illinois where we conducted our research, *T. tetrophthalmus* is univoltine and adults are present from early June to August (Price and Willson, 1976; personal observation). They are active from morning ($\approx$0800 h) through late afternoon, with few flying at dusk and none at night (personal observation). Adults live for about 40 days, and both sexes mate multiple times (Matter *et al.*, 1999).

Adult *T. tetrophthalmus* are relatively sedentary and rarely disperse from or between milkweed patches (McCauley *et al.*, 1981; Lawrence, 1982). Thus, they tend to mate and oviposit in their natal patch, giving rise to genetic structuring of populations at the level of the milkweed patch (Eanes *et al.*, 1977; McCauley, 1991). Sex ratios within patches vary from female- to male-biased (Lawrence, 1986, 1988). Males are more inclined to disperse from patches when the sex ratio is male-biased and mating success is low (McLain and Boromisa, 1987; Lawrence, 1988).

Within patches of milkweed, adult *T. tetrophthalmus* commonly aggregate on individual stems, with as many as 13 beetles per stem (Chemsak, 1963; Hartman, 1977; McCauley, 1982). Chemsak (1963) suggested that adults aggregate because they prefer individual plants within patches, while Hartman (1977) suggested that beetles are attracted by conspecifics on plants and consequently tend to aggregate. In this study, we examined ecological and behavioral factors involved in aggregation of *T. tetrophthalmus* at the spatial scale of the individual milkweed stem. First, we evaluated residence time and dispersion within the patch with mark–recapture studies. We tested the hypothesis that aggregation is cued by the host plant by removing groups of beetles from milkweed stems and subsequently monitoring aggregation for consistency in selection of hosts. To determine whether aggregation was associated with milkweed inflorescences, we tested for a correlation between numbers of umbels and beetle density per plant. To test the hypothesis that attraction to stems is determined by inflorescence size, we manipulated umbel size and subsequently monitored aggregation. We also tested the hypothesis that aggregation was influenced by the presence of conspecifics by manipulating the abundance and sex ratio of beetles on stems and recording the number and sex of arriving beetles. To identify sensory cues involved in aggregation (vision versus olfaction), we observed the response of beetles to caged and uncaged conspecifics on milkweed stems.

MATERIALS AND METHODS

Study Sites

We conducted field research on *T. tetrophthalmus* during the summers of 1997 through 2001 at Phillips Tract (PT), a University of Illinois natural area in Champaign County. PT is a 52-ha former farm containing old-field areas dormant for at least 1 year prior to our study but mowed every spring. There were several patches of milkweed of varying size in the old-field areas. We also conducted fieldwork during the summer of 2000 at Rutan Research Area (RRA) in Vermilion County, IL, ≈21 km from PT. RRA is a 10-ha open upland woodlot owned by the University of Illinois, with patches of milkweed at the forest edge.

Residence Time and Dispersion Within Milkweed Patches

We examined the residence time of *T. tetrophthalmus* within a milkweed patch at PT by capturing beetles within one patch of ≈900 stems between 22 June and 9 July 1997, individually marking them on the pronotum and elytra using a paint-color and dot-position scheme (Testor's® enamel paint; Phoenix Model Co., Brooksville, FL), and releasing them at the collection site immediately after marking. Beetles were chilled on ice for about 1 min to facilitate marking. Between 7 and 18 July, we searched for marked beetles in the original milkweed patch and in nearby patches and measured the distance they had moved since release.

We also conducted a mark–recapture study at RRA in summer 2000 to evaluate residence time, sex ratio, dispersion within the patch, and aggregation behavior. This study was conducted in a single milkweed patch of 85 stems that was ≈375 m^2 in area and located along a forest edge, ≈100 m from the nearest milkweed patch. On 28 June, beginning at 0800 h (when most beetles were still inactive), we collected all the beetles in the patch from milkweeds and nonhost vegetation (primarily goldenrod and other forbs and grasses) and placed them individually into 20-ml glass vials. In the laboratory, we sexed beetles and marked them with a spot of paint: Beetles from aggregations were marked with a white dot on the elytra, with the dot position indicating the aggregation from which they had been collected. We defined an aggregation as four or more beetles because such groupings were rare and so represented a behavioral extreme. Beetles not associated with aggregations were marked with either a white dot on the pronotum (if on milkweed) or a yellow dot (if on nonhost vegetation). Marked beetles were returned to RRA in a 0.3-m^3 cage of aluminum window screen and released within a few hours of capture by opening the cage on nonhost vegetation near the center of the milkweed patch. Most beetles walked out of the cage onto vegetation rather than dispersing by flying. Beginning at 0800 h on 29 June, 30 June, and 4 July (24, 48, and 144 h after release), we searched the patch for beetles and recorded the number of beetles, and their paint markings, if any. With these data we calculated the proportion of beetles that remained in the patch over time.

Role of Plant Characteristics in Aggregation

To evaluate the role of the host plant in *T. tetrophthalmus* aggregation, we removed groups of beetles from a patch of 291 milkweed stems at PT on 1 July 1998, and from another patch of 238 milkweed stems in PT on 13, 14, and 22 July 1999, and subsequently monitored aggregation. Milkweed stems were labeled with plastic tags, and beetles were marked with a dot of paint with color and position indicating the stem on which they were first captured. We released marked beetles on nonhost vegetation near the center of patches within 2.5 h of capture and checked for new aggregations in the patch after 24 and 48 h. Repeated aggregation on the same stems would be evidence that beetles were responding to qualities of individual stems or localized environmental cues. A lack of reaggregation on the same stems, however, could result from either beetles not responding to plant qualities or there being a high availability of suitable stems in the patch. In a similar study at RRA on 28–30 June and 4 July 2000, we monitored aggregation 24 h after releasing marked beetles that had been collected in the patch of

85 stems (see previous section). At both sites and in all years we estimated the population size of *T. tetrophthalmus* in the vicinity of milkweed patches using the Jolly–Seber stochastic method based on the number of marked and unmarked beetles captured 24 h after release (Southwood and Henderson, 2000).

Influence of Milkweed Inflorescences on Aggregation

Adults of some cerambycid species are attracted to host plants that have the largest inflorescences (Harman and Harman, 1987) and, presumably, offer the greatest floral resources. We tested the hypothesis that aggregation of *T. tetrophthalmus* is cued by the floral display of milkweed stems by comparing the number of beetles per stem with the number of umbels per stem in the patch of 238 milkweed stems at PT on 5 days between 14 and 22 July 1999. We excluded umbels which had senesced (evidenced by wilting and browning) because we rarely observed beetles on such umbels.

To determine whether umbel size and position influenced attractiveness of stems to beetles, we compared the number of beetles per stem with the diameter of each umbel and the leaf axil from which it arose for each of the 85 milkweeds in the patch at RRA on 28, 29, and 30 June and 4 July 2000. Because there was considerable variation among umbels in the spread of their florets, we standardized our measure of umbel size by gently compacting florets into a circle and measuring its diameter. To study the effect of senescence on the attractiveness of umbels to beetles, we measured with a protractor the obtuse angle between the dorsal side of the pedicel and the nearest edge of the umbel (this angle is $\approx 90^\circ$ in fresh umbels but increases as umbels senesce and droop) and compared this angle with the number of beetles we collected from each umbel 2 and 6 days later.

To confirm the influence of umbel size on beetle aggregation, we manipulated the umbel diameter and tested for differences in the rate at which beetles arrived on stems. We removed all umbels from 20 milkweed stems of similar height within a different milkweed patch at PT than was used in previous studies. We attached a 1-dram vial filled with water to the topmost axil of each stem with wire and placed cut, flowering umbels into the vials to form clusters of florets with diameters of ≈ 1, ≈ 3, or ≈ 6 cm when compacted ($N = 5$ stems per treatment per day). Five stems with vials containing only water served as controls. Treatments were randomly assigned to stems. We checked stems for beetles at 1-h intervals between 0900 and 1600 h. The study was replicated on 7, 10, and 13 July 2000.

Role of Conspecifics in Aggregation

We studied how the abundance of adult *T. tetrophthalmus* and their sex ratio on individual milkweed stems influenced subsequent aggregation by creating artificial groupings of beetles, preventing dispersal by gluing the elytra of beetles with a spot of Super Glue.® Glue did not appear to influence longevity or mating behavior. Beetles with glued elytra that walked off the stem were either returned to the stem or replaced with another glued beetle of the same sex. We created groups of two or six beetles to evaluate density effects on aggregation. Groupings were arbitrarily assigned to milkweed stems of similar height and number of umbels at PT, and the experiment was replicated on different days with new stems selected when umbels began to senesce. Sex ratio and beetle density treatments were as follows:

(1) six beetles on three milkweed stems, with sex ratios of 6 F:0 M, 0 F:6 M, and 3 F:3 M, conducted between 1030 and 1530 h on 9 days from 28 July to 8 August 1997; and
(2) six or two beetles on six stems, with sex ratios of 6 F:0 M, 0 F:6 M, 3 F:3 M, 2 F:0 M, 0 F:2 M, and 1 F:1 M, conducted between 1030 and 1645 h on 10 days from 9 to 23 July 1998.

At 15-min intervals, we recorded the number and sex of beetles that had arrived at each stem, removed them, and allowed them to disperse by flying. It is likely that we missed individuals that visited and left stems between observations and, therefore, measured the accumulation of beetles on stems at the end of the time interval rather than the total number of visitors.

To assess better the response of beetles to conspecifics present on stems, we conducted an independent experiment in which we continuously observed artificial groupings of beetles with glued elytra with sex ratios of 6 F:0 M, 0 F:6 M, and 3 F:3 M on individual milkweed stems ($N = 6$ stems per treatment) and recorded the amount of time that individual beetles remained on stems. As we could not determine the sex of visitors without disturbing them, visiting beetles were not sexed. We arbitrarily assigned treatments to milkweed stems of similar height and number of umbels and alternated treatments between these stems on different days. This study was conducted between 1000 and 1530 h on 5 and 6 July 2001.

Senses Involved in Aggregation and Mate Location

To identify the senses that milkweed beetles used in locating conspecifics, we evaluated the response of beetles to caged and uncaged females in the field. Because we had observed that males moved between stems much more frequently than females (see Results), and concluded that mating depends on the searching behavior of males, females were caged on stems. To provide both visual and olfactory cues, we glued the elytra of six females to prevent dispersal and placed them on a milkweed stem. Six other females with glued elytra were caged to provide olfactory cues but reduced visual cues (cages were cylinders of aluminum window screen 9 cm in diameter and 12 cm tall with plastic petri dishes at the top and bottom). An empty cage served as a control, providing neither visual nor olfactory cues associated with beetles. We were not able to develop an effective method for excluding olfactory cues but retaining visual cues; for example, the appearance of dead beetles quickly changed after death, rendering them unacceptable as models. Treatments were arbitrarily assigned to three adjacent milkweed stems of similar height and number of umbels, with new stems selected when umbels began to senesce. We observed the study plants continuously, recording the number of beetles arriving on each stem and the duration of their visits, but could not determine their sex without disturbing them. We replicated the experiment at PT between 0900 and 1600 h from 31 July to 3 August 1998 and for 11 days between 22 June and 19 July 1999.

Statistics

We used analysis of variance (ANOVA) to test differences among treatment means, after first confirming homogeneity of variances among treatments ($F_{\max}$ test [Sokal and Rohlf, 1995]); data not meeting this assumption were analyzed with the nonparametric Kruskal–Wallis test (Sokal and Rohlf, 1995; PROC NPAR1WAY, SAS Institute, 2001). Insignificant interaction terms were eliminated from ANOVA models. Differences between individual means were tested with the Ryan–Einot–Gabriel–Welsch multiple-range test (REGWQ [SAS Institute, 2001]). Probabilities of repeated aggregation of beetles on milkweed stems were calculated using the hypergeometric distribution (Sokal and Rohlf, 1995). We present means ± 1 SE, except where stated otherwise.

RESULTS

Residence Time and Dispersion Within Milkweed Patches

In the residence time study at PT, we captured, marked, and released 243 *T. tetrophthalmus*, with a male-biased sex ratio of 0.77 female/1 male (significantly different from 1/1; $\chi^2 = 3.95$, $P < 0.05$). We recaptured only 10 males and 6 females (4.1 and 2.5% of the total number of beetles released) after an average of 5.56 ± 1.1 days (±SD).

At RRA, we collected 184 beetles in a milkweed patch of 85 stems in early morning; 75% of the beetles were on milkweeds and 25% on other plant species. The sex ratio was significantly male-biased, with 0.68 female/1 male (significantly different from 1/1; $\chi^2 = 6.57$, $P < 0.025$). The sex ratio did not differ significantly for beetles on milkweed stems versus other plant species ($\chi^2 = 1.74$, $P > 0.05$), suggesting that the sexes did not differ in plant species they selected to spend the night on. The sex ratio of beetles on individual milkweed stems averaged 0.61 female/1 male and was not correlated with the beetle density per stem (Pearson's $r^2 = 0.0017$, $N = 38$, $P = 0.34$), suggesting that the sexes also did not differ in their inclination to aggregate at the end of the day. We marked and released 105 male and 71 female beetles. The number of beetles in the patch declined sharply by 24 h after the release of marked beetles (Fig. 1, inset) but remained fairly steady during the subsequent 2 days. Marked beetles rapidly disappeared from the patch, with few remaining after 2 days (Fig. 1).

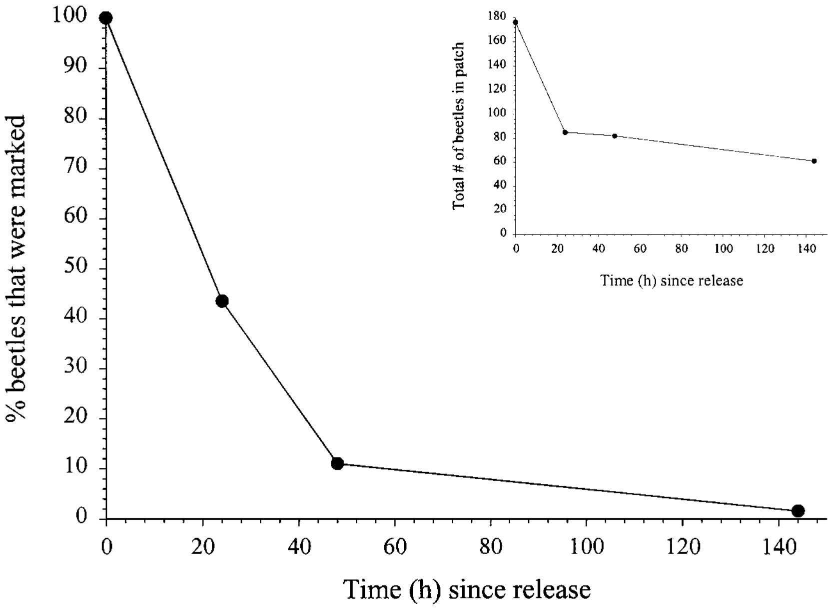

Fig. 1. Percentage of marked beetles in the patch at RRA over a 6-day period. Inset: Change in total population size within the patch over time.

Role of Plant Characteristics in Aggregation

At PT in 1998, we estimated the local population size of *T. tetrophthalmus* to be 826 beetles in the patch of 291 stems. After we removed aggregations of beetles from five milkweed stems, no new aggregations formed by the next day, but on the second day beetles had aggregated on six stems, including three of the original stems; none of these beetles had been present in the original aggregations. The probability of these aggregations reoccurring by chance on three of the five original stems in a patch of 291 stems was $\approx 1/(2 \times 10^6)$.

In the 1999 study at PT, we estimated the population size to be 663 beetles in the patch of 238 stems. Beetles did not reaggregate on two milkweed stems in the patch of 238 stems but aggregated on two other stems within 48 h.

At RRA in 2000, we estimated the population size to be 406 beetles in the patch of 85 stems. Beetles aggregated within 24 h on 1 stem that originally did not have an aggregation and on 7 of 15 stems from which we had removed aggregations. The probability of beetles reaggregating by chance on 7 of 15 stems is $\approx 1/(1 \times 10^7)$. Only 23% of beetles in new aggregations had been marked, and of these, 65% were first collected from milkweed and 35% from other plant species ($\chi^2 = 1.8$, $P > 0.1$), suggesting that aggregation was not associated with the type of plant from which beetles had originally been collected. The findings of these reaggregation studies strongly suggest that beetles do not randomly select stems within a patch for aggregation but, rather, tend to aggregate consistently on certain individual stems within a milkweed patch.

Influence of Milkweed Inflorescences on Aggregation

At PT in 1999, there was an average of 0.97 ± 1.3 (SD) umbels per milkweed stem in the patch of 238 stems. We observed 631 *T. tetrophthalmus* over a 5-day period with a mean density of 0.33 ± 0.77 (SD) beetles per stem; most stems did not have any beetles (Fig. 2A). Densities of adult beetles per stem were positively associated with the number of umbels per stem (Fig. 3): Stems with one umbel had more than five times more beetles than stems without umbels, and this number nearly doubled on stems with three and four umbels (means significantly different; overall ANOVA, $F = 11.2$, $\mathrm{df} = 4, 1150$, $P < 0.0001$; umbel effect, $F = 57.2$, $P < 0.0001$; day effect, $F = 7.51$, $P < 0.0001$).

In the milkweed patch of 85 stems at RRA, there was an average of 1.56 ± 2.3 (SD) umbels and 1.6 ± 2.5 (SD) beetles per stem, with 15

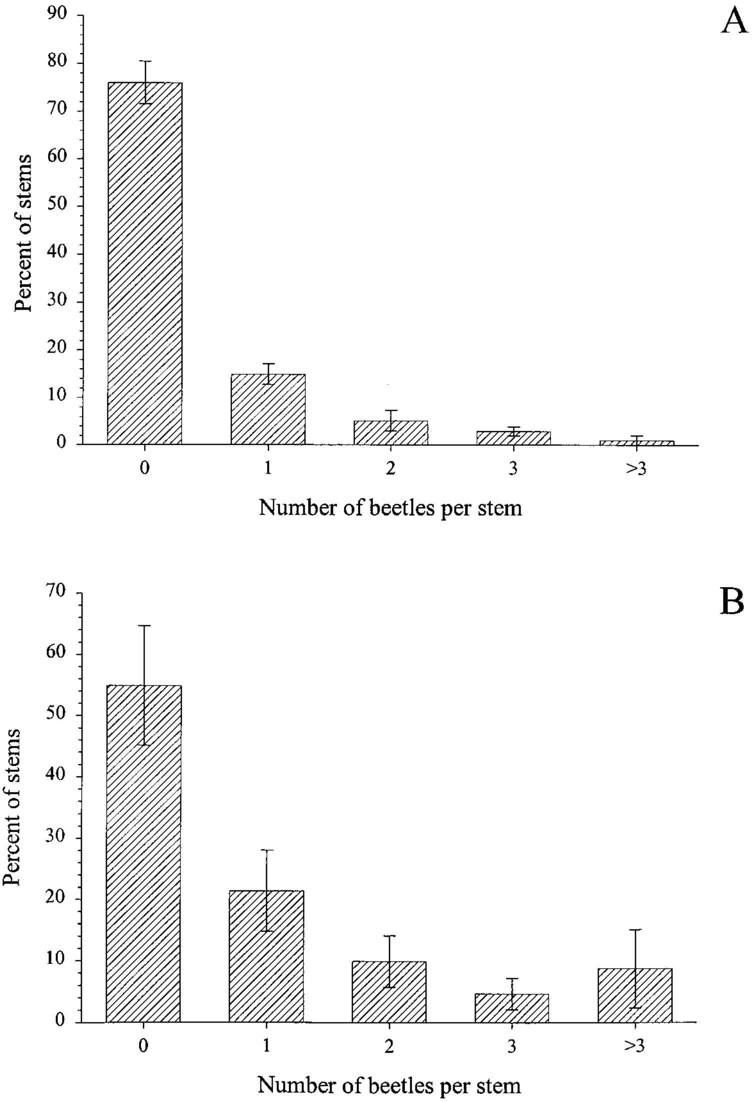

Fig. 2. Frequency distribution of the number of *T. tetrophthalmus* adults per milkweed stem (mean ± 1 SD) at (A) PT ($N = 5$ days) and (B) RRA ($N = 4$ days).

aggregations of four or more beetles. As at PT, most stems had no beetles (Fig. 2B). Also consistent with the PT study, beetles were more abundant on stems having umbels than on stems without umbels (means, 1.72 ± 0.19 and 0.57 ± 0.09 beetles per stem, respectively; overall ANOVA, $F = 11.9$,

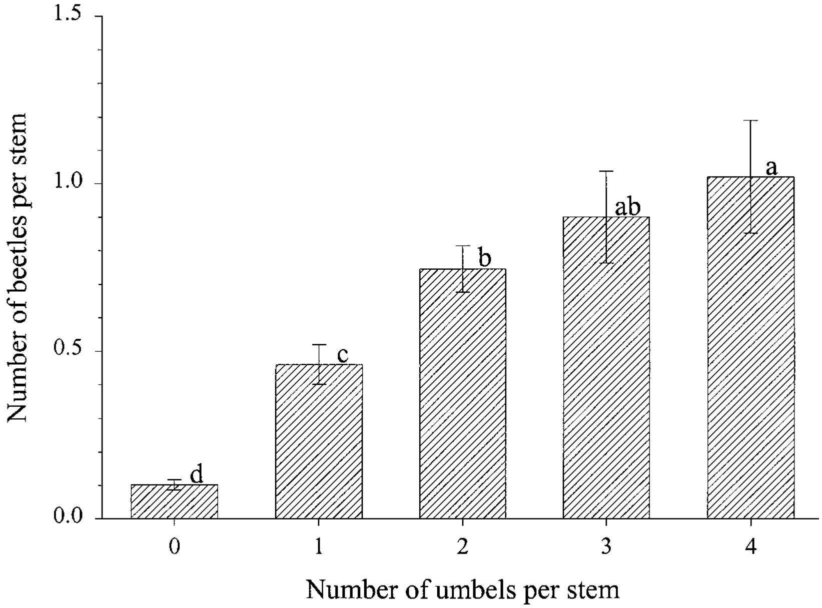

Fig. 3. Relationship between number of milkweed umbels and number of *T. tetrophthalmus* adults per stem (mean ± 1 SE). Sample sizes for zero, one, two, three, and four umbels per stem were 635, 195, 220, 75, and 50 plants, respectively. Means with different letters were significantly different (REGWQ test, $P < 0.05$).

df = 4, 335, $P < 0.0001$; umbel effect, $F = 34.5$, $P < 0.0001$; day effect, $F = 4.32$, $P = 0.0053$) and about 17 times more abundant on umbels on the topmost axil than on those of the second or third axils, averaging 0.51 ± 0.1, 0.03 ± 0.03, and 0.02 ± 0.02 beetles per umbel, respectively (means significantly different; Kruskal–Wallis statistic = 47.6, df = 2, 275, $P < 0.0001$). Fresher umbels on upper axils were more erect than those on lower axils, with a smaller angle between the dorsal side of the pedicel and the nearest edge of the umbel (Fig. 4; means significantly different; ANOVA, $F = 86.6$, df = 1, 122, $P < 0.0001$). The average angle of umbels from which beetles were collected was 116 ± 55° (SD), which fell within the 99% confidence interval for umbels on the upper two axils, further demonstrating that beetles responded to fresher apical umbels.

The number of beetles on milkweed stems increased significantly with the diameter of umbels on the upper two axils, increasing two- to fivefold as the umbel diameter exceeded 3 and >6 cm, respectively (Fig. 5; means significantly different; overall ANOVA, $F = 3.53$, df = 3, 135, $P < 0.0001$). In fact, the stems on which beetles repeatedly aggregated (on the first and second days) had significantly larger umbels on the upper two axils than did

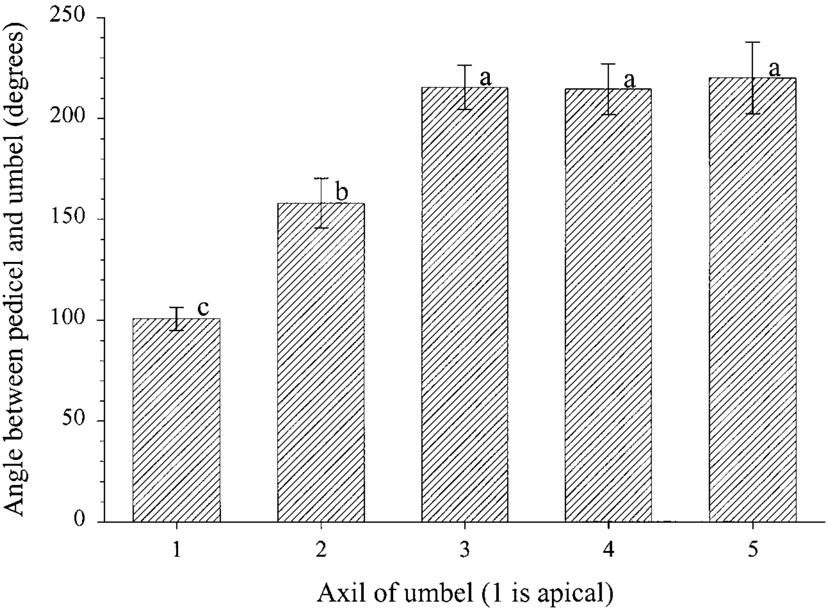

Fig. 4. Relationship between the axil of the milkweed stem from which umbels arose and the angle between the umbel and the pedicel (a measure of senescence). Sample sizes were 45, 31, 25, 16, and 7 stems, respectively. Means with different letters were significantly different (REGWQ test, $P < 0.05$).

other stems in the patch (means, 6.61 ± 1.2 and 2.4 ± 0.4 cm, respectively; ANOVA, $F = 9.1$, df = 1, 84, $P = 0.0034$). The beetle density per stem also increased with the basal diameter of milkweed stems (Pearson's $r^2 = 0.205$, $N = 334$, $P < 0.0001$), which was autocorrelated with the summed diameter of umbels on the upper two axils (Pearson's $r^2 = 0.336$, $N = 334$, $P < 0.0001$). The influence of inflorescence size on aggregation was confirmed by the more than twofold increase in numbers of beetles that visited milkweed stems to which we had attached artificially-created umbels ≈6 cm in diameter compared to stems with smaller or no umbels (means for plants with no umbels and umbels with diameters of 1, 3, and 6 cm: 0.13 ± 0.05, 0.13 ± 0.04, 0.14 ± 0.04, and 0.36 ± 0.07, respectively; Kruskal–Wallis statistic = 11.8, df = 3, 376, $P = 0.0082$).

Role of Conspecifics in Aggregation

Of beetles arriving at artificial groupings of beetles, 44 of 52 (85%) were males in the 1997 study and 104 of 112 (93%) were males in the 1998 study

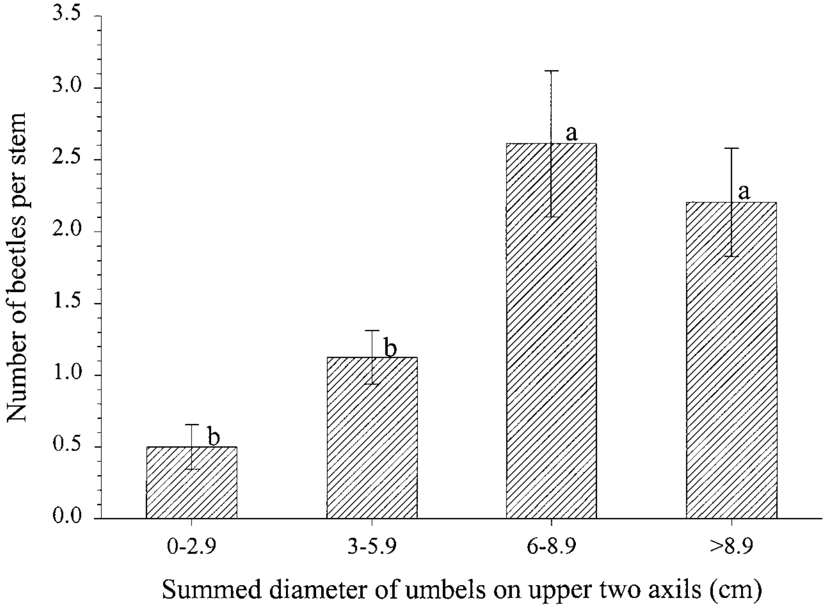

Fig. 5. Relationship between the summed diameter of umbels on the upper two axils of milkweed stems and the number of *T. tetrophthalmus* per stem at RRA. Sample sizes from smallest to largest categories were 32, 40, 36, and 44 stems. Means with different letters were significantly different (REGWQ test, $P < 0.05$).

(sex ratios significantly different from 1/1; $\chi^2 = 18.8$, $P < 0.001$, and $\chi^2 = 47.7$, $P < 0.001$, respectively). These findings suggest that males are much more likely to move between milkweed plants than females. We observed more beetles on female-biased groupings of six beetles than male-biased groupings in 1997 (Fig. 6A; means significantly different; overall ANOVA, $F = 3.95$, df = 2, 26, $P = 0.0128$; sex effect, $F = 4.0$, $P < 0.04$; day effect, $F = 3.48$, $P < 0.018$). The 1998 study showed a similar pattern, with more beetles on stems having female-biased groupings of six beetles (Fig. 6B; overall ANOVA, $F = 8.07$, $P < 0.0001$; sex effect, $F = 6.06$, $P = 0.0002$; day effect, $F = 8.56$, $P < 0.0001$), however, treatment means were not significantly different for groupings of only two beetles (REGWQ test, $P > 0.05$). When we continuously observed groupings of beetles, however, the number of beetles arriving on stems was not influenced by the sex ratio (Fig. 7, bars; overall ANOVA, $F = 0.17$, df = 2, 12, $P = 0.97$), although beetles remained for longer periods on stems with six females than on stems with no females (Fig. 7, dots; means significantly different; ANOVA sex effect, $F = 3.85$, df = 2, 83, $P = 0.025$). These findings suggest that the sex ratio effect in the first study was probably due to accumulation of visiting beetles at female-biased aggregations rather than differences in rates of arrival across treatments.

Senses Involved in Aggregation and Mate Location

Similar numbers of beetles arrived at milkweed stems having an empty cage, six caged females, and six uncaged females with glued elytra (Fig. 8, bars; means not significantly different; overall ANOVA, $F = 1.41$, df = 2, 14, $P = 0.25$). However, visitors stayed on milkweeds with uncaged females nearly twice as long as on stems with caged females or no females (Fig. 8, dots; means significantly different; overall ANOVA, $F = 8.59$, df = 2, 189, $P < 0.0001$; treatment, $F = 28.0$, $P < 0.0001$; day effect, $F = 14.7$, $P < 0.0001$). The significant treatment × day interaction term ($F = 3.82$, $P < 0.0001$) was due to the small sample size on certain days. These findings further suggest that the chances of males arriving at any particular stem were not influenced by the presence of females, but males remained for longer periods, and so tended to accumulate, on stems where they encountered females.

DISCUSSION

Our studies failed to demonstrate the use of a long-range sex attractant by adult *T. tetrophthalmus*. Instead the sexes appeared to be brought together by a mutual attraction to milkweed stems, where they feed on inflorescences and foliage. Mate location in many other species of longhorned beetles depends on behavioral cues other than long-range pheromones, including aggregation in response to plant volatiles (Hanks, 1999; Ginzel and Hanks, 2002b).

The low recapture rates in our residence time study suggest that many beetles of both sexes dispersed from the patch or died before recapture. It seems unlikely that marking beetles influenced their longevity because the same type of paint (Testor's® enamel) has been used in field and laboratory studies of *T. tetrophthalmus* and other cerambycid species without apparent ill effects (e.g., Hanks *et al.*, 1998; Ginzel and Hanks, 2002a). Moreover, marked beetles recovered several weeks after release appeared to behave normally. It also seems doubtful that paint-marking would have increased the probability of predation because *T. tetrophthalmus* is aposematically colored and generally avoided by birds (Jones, 1932), although the amount of cardiac glycosides in *T. tetrophthalmus* may not be great enough to deter predation by vertebrates (Isman *et al.*, 1997).

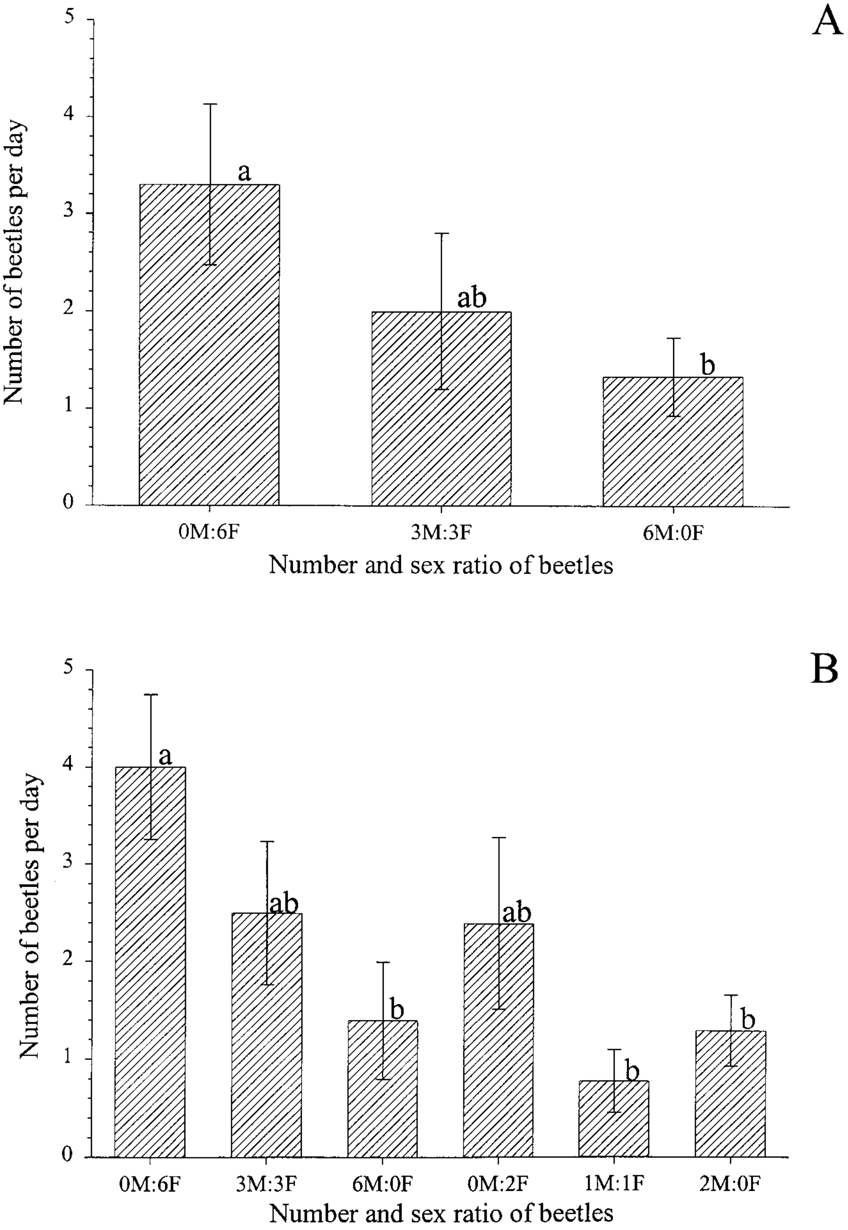

Fig. 6. Relationship between the sex ratio of *T. tetrophthalmus* in artificial groupings of (A) Six beetles in 1997 ($N = 3$ stems on each of 9 days) or (B) six and two beetles in 1998 ($N = 6$ stems on each of 10 days) and the mean number of beetle visitors observed on milkweed stems. Means with different letters were significantly different (REGWQ test, $P < 0.05$).

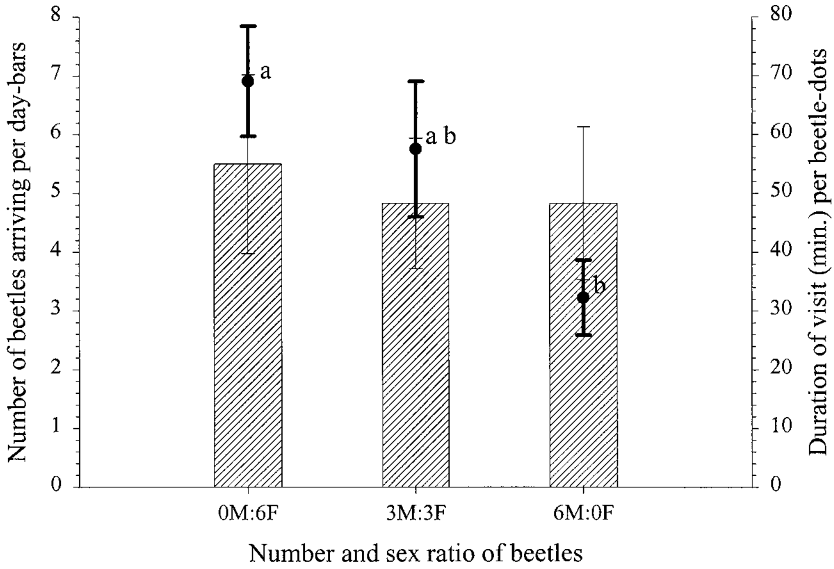

Fig. 7. Relationship between the sex ratio of *T. tetrophthalmus* adults in artificial groupings and the mean number of beetles arriving on milkweed stems during continuous observation (bars; $N = 9$ stems on each of 2 days) and the duration of their visits (dots with error bars; $N = 26$–36 beetles per treatment). Means with different letters were significantly different (REGWQ test, $P < 0.05$).

The dynamic structure of *T. tetrophthalmus* populations was also evident in our mark–recapture study at RRA. Of 176 marked beetles we released, only 37 (≈21%) were still present in the patch 24 h later, although there was a total of 82 beetles due to the appearance of unmarked individuals (Fig. 1, inset). These unmarked beetles may have eclosed within the patch, but it seems unlikely that recently emerged beetles would comprise such a large proportion of the population in only 24 h. Beetles immigrating into the patch may have originated from another patch 100 m away or from greater distances. Marked beetles may have fled the patch during the first 24 h (Fig. 1) in response to handling, but we doubt that handling would have encouraged dispersal during subsequent days. We therefore conclude that the nearly complete replacement of marked beetles with unmarked newcomers within 6 days suggests that beetles were highly mobile and rates of immigration and emigration were high. Mark–recapture studies at both sites suggest that considerable numbers of beetles emigrated and immigrated at the spatial scale of the milkweed patch, a finding inconsistent with earlier studies that suggest that *T. tetrophthalmus* adults are disinclined to disperse among milkweed patches (McCauley *et al.*, 1981; Lawrence, 1982; McCauley, 1983; McCauley and Eanes, 1987).

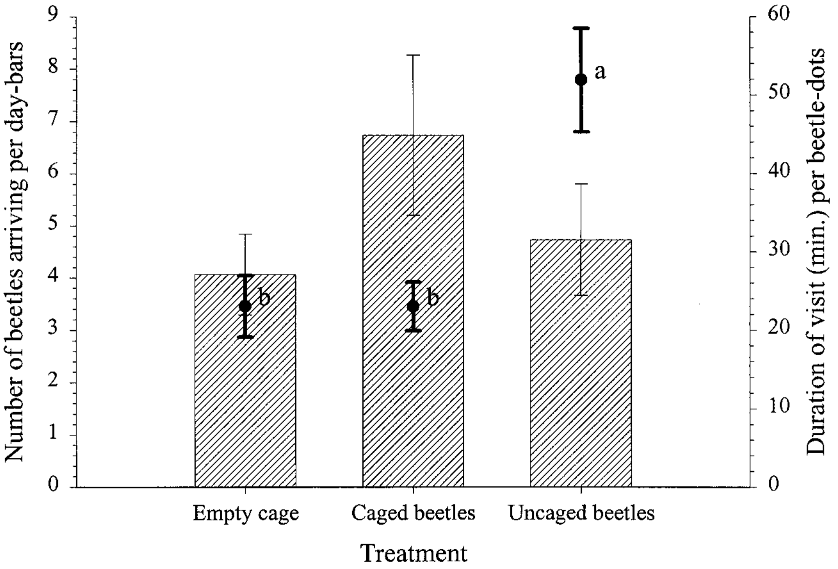

Fig. 8. Relationship between caging treatments of six female *T. tetrophthalmus* on milkweed stems and the proportion of beetles arriving (bars; $N = 1$ stem per treatment each of 15 days) and the duration of their visits (dots with error bars; $N =$ 70–101 beetles per treatment). Means with different letters were significantly different (REGWQ test, $P < 0.05$).

Beetles were unevenly distributed within milkweed patches, with most stems having no beetles and only a few having aggregations. When we removed aggregations from individual stems, beetles often reaggregated on some of the same milkweed stems, confirming that aggregations did not arise randomly and suggesting that beetles were cued by qualities of individual host plants. There remains the possibility that the attractiveness of plants was altered by the original beetle aggregations, such as by their feeding on plant tissues or depositing some chemical cue. Beetles tended to aggregate on stems that had umbels, and among those stems they preferred larger, fresh umbels on upper axils. The importance of the inflorescences in attracting. *T. tetrophthalmus* accounts for the observation by Matter *et al.* (1999) that adults were less abundant in areas of milkweed patches from which umbels had been removed. Our study further revealed that different beetles within populations aggregated on different days and on different milkweed stems. This finding suggests that, although the location of aggregations within a milkweed patch often is not random with regard to host plant, beetles that participate in aggregating appear to be a random sample of the population.

Most of the beetles arriving at our artificially created aggregations were male, probably because male *T. tetrophthalmus* tend to be more active than females (McCauley *et al.*, 1981). This phenomenon has also been observed in other cerambycid species (Hanks, 1999). Males tended to accumulate in aggregations that were female-biased, probably due to the prolonged copulatory period of this species ($\approx$3 h [McCauley and Lawson, 1986]). However, neither the presence of conspecifics on stems nor the sex ratios of aggregations influenced the rate at which beetles, primarily males, arrived on stems, suggesting that they moved arbitrarily between stems that had similar floral displays. Our study with caged and uncaged beetles also showed that beetles moved between stems independent of conspecifics, further attesting to an absence of either visual or olfactory cues that operated over a distance and demonstrating that males accumulated on stems where they had access to females.

We conclude that aggregation of *T. tetrophthalmus* is cued by host plant characteristics and dynamically influenced by the sex ratio of conspecifics already present on individual stems. Males tend to accumulate on stems that have available females, and this behavior appears to stabilize the male-biased sex ratio of aggregations.

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