Biology and Reproductive Behavior of *Murgantia histrionica* (Heteroptera: Pentatomidae)

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ABSTRACT Life history parameters and reproductive behaviors of the harlequin bug, *Murgantia histrionica* Hahn (Heteroptera: Pentatomidae), were determined. Total developmental time from egg to adult was ≈48 d. After a sexual maturation period of ≈7 d, both sexes mated repeatedly, with females laying multiple egg masses of 12 eggs at intervals of 3 d. Adult females lived an average of 41 d, whereas adult males lived an average of 25 d. Courtship and copulation activities peaked in the middle of the photophase. In mating experiments in which mixed sex pairs of virgin and previously mated bugs were combined in all possible combinations, the durations of courtship and copulation by virgin males were significantly longer with both virgin and previously mated females than the same behaviors for previously mated males. When given a choice between a virgin or previously mated female, previously mated males preferred to mate with virgin females, whereas virgin males showed no preference for virgin over previously mated females. Analyses of mating behaviors with ethograms and behavioral transition matrices suggested that a primary reason for failure to copulate by virgin males was the incorrect rotation of their pygophores to the copulation position, so that successful alignment of the genitalia could not occur.

KEY WORDS harlequin bug, Hemiptera, Pentatomidae, courtship, behavioral sequences

Historically, the harlequin bug, *Murgantia histrionica* Hahn (Heteroptera: Pentatomidae), has been one of the most destructive insects of cole crops in the United States (Brett and Sullivan 1974). Despite its economic importance, the biology and reproductive behaviors of harlequin bugs have not been subjected to detailed study. For example, Canerday (1965) reported basic biological information relating to the success of laboratory rearing. Lanigan and Barrows (1977) studied courtship, copulation, polygyny, and polyandry in *M. histrionica*, but their descriptions of experiments lacked important details (vide infra), and they were carried out with limited numbers of individuals of unknown (or unstated) age and sexual maturity. In preliminary studies of courtship and copulation, using sexually mature virgin *M. histrionica*, we found that the copulation and courtship behaviors did not seem to have been accurately and completely described by previous workers. To resolve these discrepancies, we carried out detailed studies of the general life history and reproductive behaviors of *M. histrionica*, with the following specific objectives: 1) to determine general life history parameters for harlequin bugs reared under standardized conditions in the laboratory; 2) to describe and analyze the courtship and copulation behaviors of harlequin bugs; 3) to determine the diurnal cycles of courtship and copulation; 4) to determine weight changes of males and females after copulation; and 5) To determine mating preferences between virgin and previously mated bugs.

Materials and Methods

Insect Colonies. Adults and nymphs of *M. histrionica* were collected from bladderpod, *Isomeris arborea* Nutt. (Caperaceae), from one site each in Riverside and San Diego, CA. A colony was started in the laboratory at the University of California, Riverside, in 2003, and it was augmented every year with ≈100 males, females, and nymphs from both locations. Voucher specimens have been submitted to the University of California, Riverside Entomology Research Museum (UCRC 145863–145882). Insects were reared in a growth chamber at 26 ± 1°C, ≈45% RH, with a photoperiod of 16:8 (L:D) h (lights on at 0700 and off at 2300 hours) provided by fluorescent lights (two Sylvania Octron 32-W). Immature insects were held separately from adults in cylindrical plastic containers (20 by 15 cm in diameter), with two 4-cm circular holes on opposite sides of each container covered with brass screen for ventilation. Colony adults were held

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in 75- by 42- by 45-cm glass-topped wooden rearing cages with fine-mesh screening across the back. Insects of all life stages were provided with Napa cabbage, cauliflower, broccoli, and seasonal weeds for food, which was changed three times per week. After the final molt, adults were sexed and maintained individually for 14 d in 175-ml transparent ice cream cups with breathable lids and a floret of broccoli, then they were used as needed for experiments.

General Life History Experiments. Females laid egg masses consisting of 12 eggs. Twenty-five such egg masses were collected from the breeding colony on the days that they were laid. Each mass was placed in a 9-cm-diameter petri dish and observed twice daily, recording when the eggs hatched. After hatching, neonate bugs were given fresh Napa cabbage leaves daily. After the second molt, the nymphs were placed in cylindrical 20-cm-diameter by 15-cm-high containers until the final molt to adults. All bugs were assessed daily for life stage and survival. After the final molt, 60 pairs of males and females were placed in 20- by 15-cm containers to determine the premating period. Each pair initially was observed twice per d and, based upon pilot studies indicating what time of day courtship took place, pairs were then observed continually from 0800 to 1800 hours from the fifth day on, recording when each pair courted and copulated and each female laid eggs, and the number of eggs in each egg mass. Once mated, each pair was separated and then paired up again with a fresh virgin individual of the opposite sex, to prevent any possible effects caused by a male marking a previously mated female. The new pairs were then observed in the same manner, recording courtship and copulation events. This process was repeated with each of the original insects until that individual died, so that the original 60 insects were paired with virgin insects throughout their lifetimes. The number of egg masses laid and the number of eggs per mass were recorded for each female, and each egg mass was weighed. The interval between the first and second copulations was recorded. The total number of times that each of the original 60 females and males mated during their lifetimes also was recorded, along with the durations of courtship and copulation for the first five matings. These 60 pairs of insects also were used to determine what time of day courtship and copulation took place. Courtship and copulation data were analyzed with one-way analysis of variance (ANOVA), after checking that the data were normally distributed with the Kolmogorov–Smirnov test. Means were separated with Student–Newman–Keuls tests.

Weight Change after Mating. Thirty pairs of sexually mature (>10-d-old) virgin females and males were weighed to the nearest 0.1 mg, immediately before placing each pair in a 175-ml transparent ice cream cup with a breathable lid and a floret of broccoli. Each bug was marked for identification with a coded dot of acrylic paint on the pronotum before being weighed. Forty individuals of each sex were held individually in 175-ml containers as controls. The control insects were weighed three times per day at 0800, 1300, and 1800 h. All bugs were observed continuously by an observer. For the pairs that copulated, immediately after mating, the pair was separated and each individual was weighed. Thereafter, the mated individuals were held individually in their original containers, and they were weighed on the same schedule as the control bugs. The times that courtship and copulation activities took place were noted. Once a female laid her first clutch of eggs, they were removed from the cup and counted and weighed. Pairs that did not mate within 2 h were separated and added to the control set of insects.

Analysis of Behavioral Transitions in Courtship Sequences. In pilot studies of the reproductive behaviors of *M. histrionica*, bugs exhibited behaviors that apparently differed from those previously described in the literature (Lanigan and Barrows 1977). Thus, experiments were devised to describe the entire sequence of courtship behaviors in explicit detail. In addition, the behavioral sequences of naïve and experienced bugs were compared.

The first group of observations was carried out using 90 pairs of virgin (naïve) bugs, whereas the second set used 90 pairs of previously mated (experienced) bugs. All naïve bugs were 16 d old, whereas experienced bugs were mated at 14 d and then tested at 16 d posteclosion, after females had deposited the first clutch of eggs. These ages were used to ensure sexual maturity and to ensure that previously mated females were ready to mate again, respectively. For each set of observations, one male and one female were placed on the bottom of a cage made from a 15- by 10-cm-high cylinder of wire mesh with top and bottom consisting of glass petri dishes, all of which were washed, rinsed with acetone, and baked at 150°C for a minimum of 20 min between each replicate. In each cage, two broccoli florets were provided for food because in preliminary experiments, in the absence of food, bugs tended to search for food rather than interacting with one another. The bugs were watched continuously by an observer during the experiment rather than being video taped, because bugs were sometimes not visible to the camera as they moved about the cage and around the broccoli florets. Behaviors of both males and females were documented and analyzed. If males and females did not court one another within 60 min, the sequence was classed as a failed courtship. The duration of courtship and copulation was recorded for both naïve and experienced bugs.

First-order transition matrices of total frequency of transitions (i.e., moving from one behavioral step to the next) were created for all courtship sequences (Fagan and Young 1978). Self-transitions (direct repetition of a single behavior) were not recorded because their inclusion can distort the importance of transitions between behaviors (Slater and Ollason 1973, Baker and Cardé 1979). Both the total frequency of transitions and the probability of transitions, described in Girling and Cardé (2006), were used because these methods of analysis were most appropriate for these types of transition sequences. That is, using total frequency of transitions alone could result in repeated oscillation between two behaviors, contrib-

uting more to the composite sequence than an individual which performs the behavior once (Charlton and Cardé 1990). Conversely, the use of probabilities alone can result in disproportionate weight being given to rare transitions to and from rare behaviors, and the exclusion of valid transitions that are repeated by all individuals. Moreover, probability data do not meet the assumptions required for statistical analyses. Therefore, transition matrices were analyzed separately for both frequencies and probabilities, and the results were compared.

To analyze significant total frequency of transitions, a modification of Deming–Stephan iterative proportional fitting was used to produce expected values (Bishop et al. 1975) while taking into account the presence of structural zeroes, i.e., zeroes present as a result of either self-transitions or transitions that were not physically possible. The most probable behavioral transition sequence for each courtship category was determined using standard normal deviates, which were calculated for each transition using expected values created using the iterative method described above and applied to a binomial test for individual transitions (Stevenson and Poole 1976, Teal et al. 1981, Siegel and Castellan 1988, Girling and Cardé 2006).

Mating Preference for Virgin or Previously Mated Insects. These experiments were conducted with 12 sexually mature, naïve 16-d-old males paired with 16-d-old naïve females, and 12 naïve 16-d-old males paired with 16-d-old females that had previously mated once. The experiments were then repeated with 12, 16-d-old males that had previously mated once, paired with 16-d-old naïve females, and 12 previously mated 16-d-old males paired with 16-d-old previously mated females. Pairs were placed in 15- by 10-cm wire mesh cages as described above, along with two florets of broccoli. The bugs were marked with colored dots of acrylic paint to distinguish them from each other and were watched continuously by an observer during the experiments. Once courtship started, it was timed and observed. If courtship did not lead to copulation, observation continued until copulation occurred, with the time of each new courtship event recorded as a new time interval. The courtship interval was defined as the time interval from the initial contact which eventually led to copulation to the initiation of copulation. The copulation duration for each pair was recorded to see if there were differences between naïve and experienced bugs. The data on courtship and copulation durations were analyzed initially using a chi-square test to determine whether there was a difference between naïve or experienced males courting and copulating with naïve or experienced females. To analyze differences in courtship and copulation duration between naïve and experienced males, the one-way nonparametric procedure with a Wilcoxon two-sample test was used (Sokal and Rohlf 1995).

Table 1. Adult longevity and fecundity parameters of *M. histrionica* maintained under laboratory conditions (24°C, 45% RH)

Observation	Range	Mean ± SD
Duration of egg stage (d)	3–5	3.8 ± 0.8
Duration of first instar (d)	2–5	3.3 ± 1.1
Duration of second instar (d)	3–6	4.5 ± 1.1
Duration of third instar (d)	7–11	8.9 ± 1.4
Duration of fourth instar (d)	8–18	12.9 ± 2.4
Duration of fifth instar (d)	10–18	14.4 ± 2.2
Total duration, egg to adult (d)	37–57	47.8 ± 11.1
Days to first mating	5–10	6.7 ± 1.7
Days between first mating to first egg mass	1–6	2.2 ± 1.6
Days from maturity to first egg mass	6–16	9.0 ± 2.4
Period between first and second copulations	2–11	2.4 ± 2.5
No. of egg masses per female	2–14	8.9 ± 1.3
Wt of egg masses (mg)	8.4–9.0	8.7 ± 0.2
Days between egg masses	1–12	3.1 ± 1.7
Total oviposition period (d)	3–59	30.8 ± 14.1
No. of times mated, female	1–12	6.0 ± 2.4
No. of times mated, male	1–17	8.2 ± 3.8
Adult female longevity (d)	9–67	41.3 ± 14.3
Adult male longevity (d)	9–39	25.1 ± 8.2

Results are based on 60 pairs of freshly molted virgin adults.

Results

General Life History. Table 1 summarizes data on the life history parameters of bugs reared under standardized conditions at 26 ± 1°C and ≈45% RH. On average, *M. histrionica* eggs hatched in slightly <4 d, and there were five instars, with the duration of each successive instar increasing from 3.8 d for the first instar to 14.4 d for the fifth instar (Table 1). Egg viability was 100%, survivorship to the final molt was 86%, and the sex ratio of adult bugs did not differ from 1:1 (133 females and 125 males reared from 300 eggs). Neonate bugs were gregarious until the second molt, and they did not feed until after molting to the second instar (Canerday 1965). The tendency to aggregate decreased after the second molt, and second and later instars readily fed on the host material provided. The relatively small percentage of nymphs that did not survive seemed shriveled and desiccated. This effect may have been due to these bugs having damaged mouthparts, caused during handling as the bugs were moved from decaying food to fresh food before retracting their mouthparts, and presumably causing them to desiccate, starve, or both.

Parameters associated with adult longevity and reproductive behaviors are summarized in Table 1. Adult females that were paired repeatedly with virgin males lived an average of 41.3 ± 14.3 d, whereas males paired repeatedly with virgin females lived 25.1 ± 8.2 d. The sexual maturation period for females, as assessed by the period between the final molt and first mating, was 6.7 ± 1.7 d, with the first egg mass being laid 2.2 ± 1.6 d after mating. The second copulation occurred ≈2–3 d after the first. Over the course of their lifetimes, females laid 8.9 ± 1.3 egg masses spaced 3.1 ± 1.7 d apart. Each egg mass always consisted of 12 eggs, laid in two rows of six, with the weights of egg masses remaining virtually constant (8.7 ± 0.2 mg;

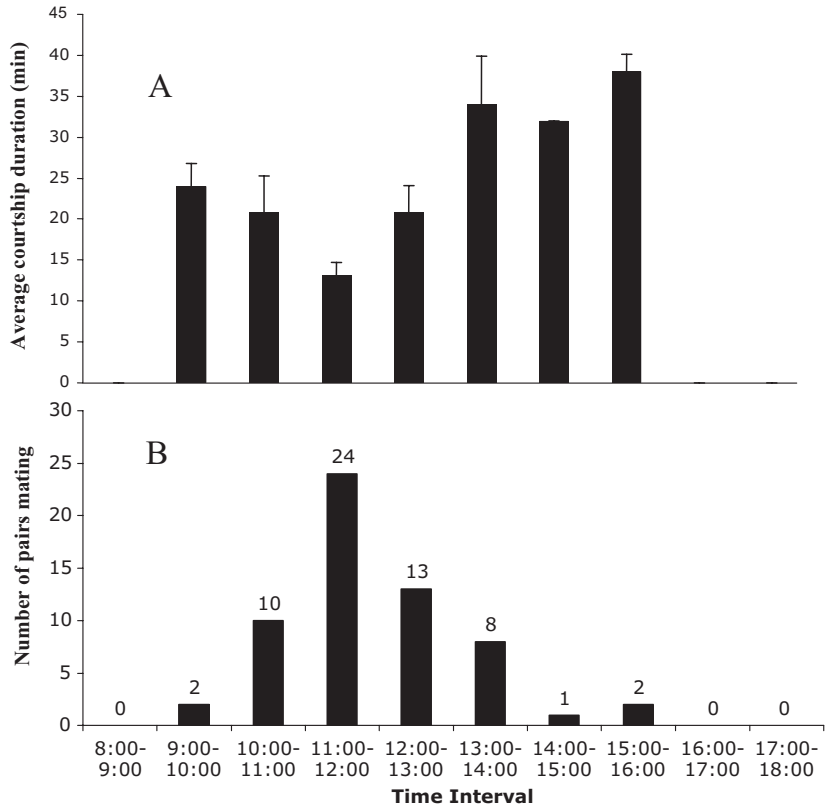

Fig. 1. Diurnal pattern of courtship activity and frequency distribution based upon 60 pairs of *M. histrionica.* (A) Average duration of courtship (minutes) at each time interval. (B) Number of pairs mating at each time interval. Photophase was from 0700 to 2300 hours.

range 8.4–9.0 mg). The total period over which eggs were laid averaged 30.8 ± 14.1 d. During their lifetimes, females paired repeatedly with virgin males mated 6.0 ± 2.4 times, whereas males paired repeatedly with virgin females mated 8.2 ± 3.8 times (Table 1).

Courtship took place only during the photophase, with courtship durations varying from <15 to >35 min. Most reproductive activity occurred from 1000 to 1400 hours (Fig. 1). When 30 males and 30 females were followed through the first five copulations with virgin partners, the duration of courtship was significantly longer for the first courtship involving two naïve bugs than for the next four courtship events in which the male was experienced and the female was unmated. The durations of the latter four events were statistically equivalent (Table 2). The duration of copulation was also significantly longer for virgin than experienced male bugs (Table 2). In contrast, the durations of courtship and copulation of either naïve or experienced females, by a succession of virgin males, were equivalent for the first five copulations (Table 2).

To determine which individual in a pair controlled the duration of courtship and copulation, further experiments were set up with all possible combinations of naïve and experienced bugs of both sexes. Naïve males were less effective at courtship than experienced males, with naïve males having to court both naïve or experienced females for more than twice as long before copulation ensued (Table 3). Further-

Table 2. Courtship and copulation duration (mean ± SE) for the first five copulations by bugs of each sex ($N = 30$) repeatedly paired with virgins of the opposite sex

Event no.	Courtship duration (min)	Copulation duration (h)
Males repeatedly paired with virgin females		
First	20.1 ± 3.15a	31.4 ± 5.08a
Second	7.8 ± 1.50b	5.0 ± 0.43b
Third	7.3 ± 0.96b	4.0 ± 1.61b
Fourth	7.0 ± 0.31b	4.6 ± 2.05b
Fifth	6.2 ± 1.42b	3.2 ± 1.48b
Females repeatedly paired with virgin males		
First	20.1 ± 0.93a	32.2 ± 0.68a
Second	19.3 ± 1.33a	22.7 ± 1.48a
Third	18.5 ± 1.30a	23.0 ± 1.30a
Fourth	20.6 ± 1.28a	21.3 ± 1.69a
Fifth	19.6 ± 1.34a	30.2 ± 0.93a

Values within a group and a column that are followed by the same letter are not significantly different based upon one-way ANOVA with means separated by Student–Newman–Keuls tests ($P < 0.05$).

Table 3. Comparison of courtship and copulation durations for virgin and previously mated *M. histrionica* (*N* = 12 each)

	Virgin female	Previously mated female
Courtship duration (min; mean ± SD)		
Virgin male	20.8 ± 3.2aA	18.8 ± 2.2aA
Previously mated male	10.2 ± 1.6aB	7.5 ± 1.5aB
Copulation duration (h; mean ± SD)		
Virgin male	31.4 ± 5.0aA	21.6 ± 1.8aA
Previously mated male	9.0 ± 2.4aB	5.0 ± 0.4aB

Significant differences within a row are indicated by different lowercase letters, whereas significant differences within a group and column are indicated with uppercase letters based upon a one-way nonparametric procedure with a Wilcoxon two-sample test ($P \leq 0.05$).

more, the courtship times for experienced males courting either naïve or experienced females were equivalent, suggesting that male experience was the deciding factor in the duration of courtship. Duration of copulation followed an analogous pattern, with naïve males having much longer copulation times with both naïve and experienced females than experienced males (Table 3). Naïve males copulated with naïve and previously mated females with equal frequency ($P = 1.0$; chi-square test) (Table 4). However, experienced males mated more frequently with naïve females than experienced females ($P < 0.02$; chi-square text).

Weight Change after Mating. Twenty-four of the 30 pairs of naïve bugs used in this experiment copulated. However, it was not possible to determine weight changes after mating because the weights of both control and test bugs fluctuated dramatically and randomly. The weights of control females changed by as much as 73% of the mean weight for the given interval between weighing periods, or by as much as 77% over the entire 8-d period that females were weighed. The weights of control males changed by as much as 91% of the mean weight for the given interval between weighing periods, and by as much as 91% over the entire 8-d period that males were weighed.

Analysis of Behavioral Transitions in Courtship Sequences. Of the 90 pairs of naïve bugs used to develop an analysis of courtship sequences, 82 pairs courted, seven pairs did not initiate courtship, and one individual in one pair died. Of those courting, 53 pairs copulated. Of the 90 pairs of experienced bugs, 70 pairs courted, six pairs did not have any courtship events, and the other 14 pairs had one or both bugs die without courtship occurring. Of those courting, 57 pairs copulated. The behaviors exhibited by the males and females are defined and coded in Tables 5 (males) and 6 (females).

Table 4. Comparison of mate choice for virgin or previously mated individuals based on proportions of the total (*N* = 12)

	Virgin female	Previously mated female	χ^2
Virgin male	0.5	0.5	1.0
Previously mated male	0.83	0.17	0.02

The χ^2 value based upon actual observations.

Table 5. Description of behaviors performed by male *M. histrionica* during courtship sequences described in the accompanying ethogram

Code for behavior	Description of behavior
NC	No physical contact between the male and female
MAFf	Male approaches the female from the front
MAFP	Male approaches the female from the rear
MA + Pyg	Male antennates the female's antennae and the male extends his pygophore
MA + Pyg P	Male antennates the female's posterior and extends his pygophore if approaching from the rear
MA A	Male antennates the female's antennae
MA S	Male moves to the side of the female and antennates the female's body wall (dorsum and venter)
MA P	Male moves behind the female and antennates her posterior
MT180° + RT	Male turns head 180° away from female posterior so they are oriented end to end (180° position), and simultaneously rotates the pygophore
PR	Pygophore rotates
PR 180°	Upon turning 180° from the female posterior, the pygophore rotates 180° so that it is inverted
PR − 180°	Upon turning 180° from the female posterior, the pygophore rotates <180°, i.e. not far enough
PR + 180°	Upon turning 180° from the female posterior, the pygophore rotates >180°, i.e., too far
MT 0° + Pyg 0°	From the end-to-end (180°) position, the male turns back to the 0° position while turning the pygophore back to its normal position
ML MetaL	While in the 180° position with pygophore rotated, the male uses his metathoracic legs to locate and touch the female posterior
MN	The male remains stationary and does nothing
ME	The male moves away from the female
PC	Pair copulates

In all courtship sequences, the male approached the female in one of two ways to initiate courtship: either by approaching the female from the front (53%), so

Table 6. Description of behaviors performed by female *M. histrionica* during courtship sequences described in the accompanying ethogram

Code for behavior	Description of behavior
NC	No physical contact between the male and female
FAMf	Female approaches the male from the front
FA A	Female antennates the male's antennae
FPA MetaL	Female pushes the male away from her posterior with her metathoracic legs
FRAb	The female raises her abdomen above the mid-axis of her body
FN	The female stands stationary and does nothing
FE	The female moves away from the male
PC	Pair copulates

that the male and female were head to head; or from the rear (26%), so that the male faced the female's posterior. In the latter cases, males usually began vigorous antennation of the female's posterior immediately upon contact. In some cases, the female also approached the male, but only from the front (21%).

Analyzing transition probabilities (Fig. 2A) and transition frequencies (Table 7), the shortest route to copulation for naïve insects seemed to be: male approached the female from the rear, male antennated the female's antennae and extended his pygophore, both sexes antennated each other's antennae, male moved to female's side and antennated the side of the thorax, male moved to female's posterior and antennated the female's abdomen (bugs are oriented head to tail), male turned 180° away from female's posterior while simultaneously rotating his pygophore 180°, male coupled his genitalia with those of the female, and the pair copulated. This sequence is a truncated version of the most probable transition sequence for courtship by naïve males. In the full sequence, there were numerous instances where the males and females cycled between two behaviors many times before progressing to the next behavior.

Unsuccessful courtship by naïve individuals often followed a different trajectory (Fig. 2B; Table 8) in which the most common sequence was as follows: male approached the female from the front, male antennated the female's antennae and extended his pygophore, female antennated the male's antennae, male antennated the female's antennae repeatedly while the female remained immobile, male moved to antennate the side of the female's thorax, male moved to female posterior and antennated the female's posterior (bugs oriented head to tail with the male's pygophore in normal position), male turned 180° away from female's posterior while simultaneously rotating the pygophore, the pygophore rotated less than or >180°, coupling of genitalia did not occur, male returned to the head to tail position while simultaneously returning his pygophore to the normal position, male antennated the female's posterior. At this point the male then repeated the cycle of rotating his pygophore > or <180°, as well as locating the female with his metathoracic legs, followed by the female pushing the male away with her metathoracic legs, resulting in a failed courtship. The key step leading to a failed courtship that was not seen in successful courtships seemed to be the improper rotation of the pygophore by > or <180° so that proper coupling of the genitalia could not occur.

There were only a few differences in the transition probabilities (Fig. 2C) and transition frequencies (Table 9) from the courtship sequences of successful naïve bugs versus experienced bugs. The fastest route to copulation for experienced insects seemed to be as follows: male approached the female from the front, male antennated the female's antennae and his pygophore extended, both sexes antennated each other's antennae, male moved beside the female and antennated her thorax, male moved to female's posterior and antennated the female's posterior, male turned 180° away from female's posterior while simultaneously rotating his pygophore 180°, the female pushed the male away with her metathoracic legs, the male returned to the head to tail position while simultaneously turning the pygophore to the normal position, male antennated the female's posterior, and the latter steps of the cycle repeated until the genitalia were coupled successfully and the pair copulated or the female moved away. Similarly, with the courtship transitions of naïve bugs, there were instances in which the bugs cycled between two behaviors many times before moving on to the next behavior.

In unsuccessful courtships by experienced male bugs (Fig. 2D; Table 10), courtship progressed through all the stages up to the point where the bugs were at 180° with the male's pygophore rotated. At this point, courtship broke down if the male failed to make further contact with the female, and she walked away before the male could couple the genitalia.

Overall, 65% of the naïve males successfully copulated, whereas 81% of the experienced males copulated ($P = 0.17$; chi-square test), indicating that overall mating success was similar under the experimental conditions, even though naïve males took considerably longer to successfully couple to females.

Discussion

In agreement with a previous report (Canerday 1965), *M. histrionica* had five instars, with the duration of each successive instar increasing from 3.7 d for the first instar to 14.4 d for the fifth instar under our rearing conditions. As noted for other phytophagous pentatomid species (e.g., Fucarino et al. 2004), the newly hatched first instars remained aggregated, and they did not feed on the host plant material. The tendency to aggregate decreased after molting, and the second instars readily fed on the host material provided. In all life stages, *M. histrionica* are typically found in loose aggregations under natural conditions (D.K.Z., unpublished data).

From general observations of the breeding colony and during experiments in which bugs were paired and observed continuously, interactions between the sexes, including courtship and copulation, peaked 3–7 h after the onset of the photophase, suggesting that this was the optimum time window in which to conduct bioassays. These results differ from the general pattern seen in most other phytophagous pentatomids, in which most mating occurs somewhat later in the day, in the late afternoon and early evening (Fish and Alcock 1973; Harris and Todd 1980; McBrien et al. 2001, 2002). The pattern of reproductive activity corresponded with the production of a male-specific pheromone by males in late morning (Zahn 2006).

We had not anticipated that the body weights of male and female *M. histrionica* would fluctuate dramatically and unpredictably, which confounded attempts to determine whether males transferred a substantial mass of nutrients to females during mating, as has been noted with other pentatomid species (Wang and Millar 1997; Ho and Millar 2001a,b). This rapid

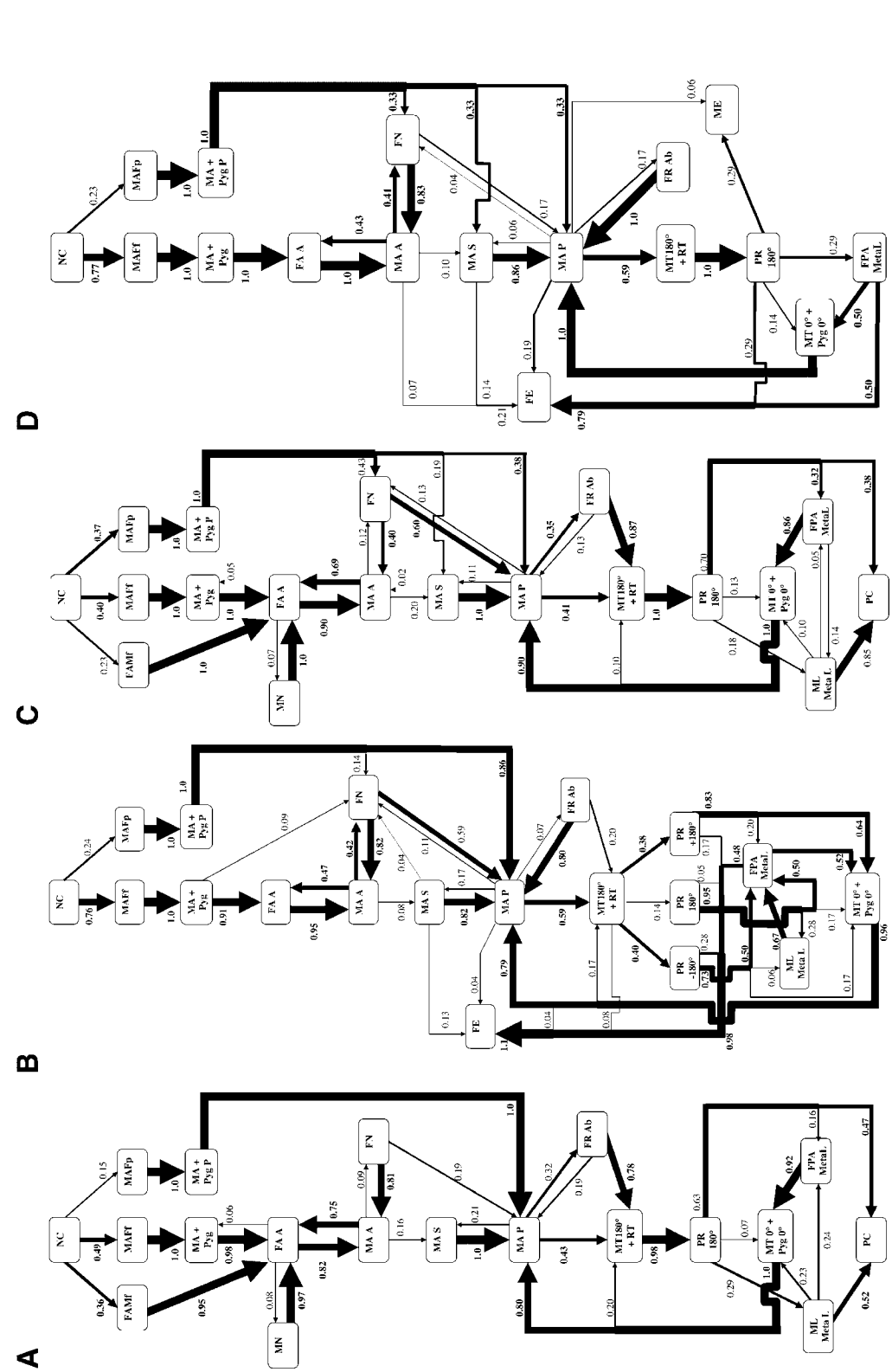

Fig. 2. Sequence of male courtship behavior observed in *M. histrionica*, constructed from mean probabilities for the following courtship categories: naïve bug successful courtship ($N = 53$) (A), naïve bug failed courtship ($N = 29$) (B), experienced bug successful courtship ($N = 57$) (C), and experienced bug failed courtship ($N = 13$) (D). In general, behaviors flow top to bottom. Transitions with a value <0.05 are not included in A, and values <0.02 are not included in B to enhance clarity of figures. Tables 5 and 6 describe the codes for the behaviors listed.

Table 7. Behavioral transition matrix for summed frequency of transitions leading to a copulation of naïve *M. histrionica* adults (*N* = 53)

Preceding behavior	Succeeding behavior																					
	FAMf	MAFf	MAFp	MA + Pyg	MA + Pyg P	MA A	FA A	FN	MN	MA S	MA P	FRA	MT180° + RT	PR 180°	PR + 180°	PR − 180°	MT 0° + Pyg 0°	ML Metal.	FPA Metal.	FE	ME	PC	Total
NC	19	*26	**8-z	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	53
FAMf	0	—	—	1	—	0	***18	0	0	—	—	0	—	—	—	—	—	—	—	0	0	—	19
MAFf	—	0	—	***26	—	0	0	0	0	—	—	0	—	—	—	—	—	—	—	0	0	—	26
MAFp	—	—	0	0	***8	—	—	0	0	0	0	0	0	—	—	—	—	0	0	0	0	—	8
MA + Pyg	—	—	—	0	—	0	***44	1	0	—	—	0	—	—	—	—	—	—	—	0	0	—	45
MA + Pyg P	—	—	—	—	0	—	—	0	0	0	***8	0	0	—	—	—	—	—	0	0	0	—	8
MA A	—	—	—	0	—	0	253	***39	0	***33	—	0	—	—	—	—	—	—	—	0	0	—	325
FA A	—	—	—	18	—	***285	0	0	***32	***11-z	—	0	—	—	—	—	—	—	—	0	0	—	346
FN	—	—	—	0	0	***40	0	0	0	0	*7-z	0	0	—	—	—	—	0	0	0	0	—	47
MN	—	—	—	0	0	0	***31	0	0	1	0	0	0	—	—	—	—	0	0	0	0	—	32
MA S	—	—	—	—	0	0	0	0	0	0	***83	0	0	—	—	—	—	—	0	0	0	—	83
MA P	—	—	—	—	0	—	—	**7	0	***38	0	***35	***65	—	—	—	—	0	1	0	0	—	146
FRA	—	—	—	0	0	0	0	0	0	0	7	0	***28	—	—	—	**1-z	0	0	0	0	0	36
MT180° + RT	—	—	—	—	—	—	—	0	0	—	—	0	0	***106	2	1	0	0	0	0	0	—	109
PR 180°	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	11	***33	***25	0	0	***37	106
PR + 180°	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	***2	0	0	0	0	—	2
PR − 180°	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	1	0	0	0	0	—	1
MT 0° + Pyg 0°	—	—	—	—	—	—	—	0	0	—	*40	0	16	—	—	—	0	—	0	0	0	—	56
ML MetaL	—	—	—	—	—	—	—	0	0	—	—	*1-z	—	—	—	—	7	0	***10	0	0	16	34
FPA MetaL	—	—	—	—	—	—	—	0	0	0	***1-z	0	0	—	—	—	***34	1	0	0	0	0	36
Total	19	26	8	45	8	325	346	47	32	83	146	36	109	106	2	1	56	34	36	0	0	53	—

Tables 5 and 6 describe the codes for the behaviors listed below. Transitions with asterisks are significantly different from expected (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Transitions indicated with -z occur less frequently than expected.

Table 8. Behavioral transition matrix for summed frequency of transitions leading to a missed copulation of naïve *M. histrionica* adults ($N = 29$)

Preceding behavior	Succeeding behavior																					
	FAMf	MAFf	MAFp	MA + Pyg	MA + Pyg P	MA A	FA A	FN	MN	MA S	MA P	FRA	MT180° + RT	PR 180°	PR + 180°	PR − 180°	MT 0° + Pyg 0°	ML Metal.	FPA Metal.	FE	ME	PC	Total
NC	0	***22	7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	29
FAMf	0	—	—	0	—	0	0	0	0	—	—	0	—	—	—	—	—	—	—	0	0	—	0
MAFf	—	0	—	***22	—	0	0	0	0	—	—	0	—	—	—	—	—	—	—	0	0	—	22
MAFp	—	—	0	0	***7	—	—	0	0	0	0	0	0	—	—	—	—	0	0	0	0	—	7
MA + Pyg	—	—	—	0	—	0	***20	2	0	—	—	0	—	—	—	—	—	—	—	0	0	—	22
MA + Pyg P	—	—	—	—	0	—	—	1	0	0	*6	0	0	—	—	—	—	—	0	0	0	—	7
MA A	—	—	—	0	—	0	82	***75	0	*12	—	0	—	—	—	—	—	—	—	**2-z	3	—	174
FA A	—	—	—	0	—	***98	0	0	***3	4	—	0	—	—	—	—	—	—	—	0	0	—	105
FN	—	—	—	0	0	***76	0	0	0	2	**10-z	0	0	—	—	—	—	0	0	1	0	—	89
MN	—	—	—	0	0	0	***3	0	0	0	0	0	0	—	—	—	—	0	0	0	0	—	3
MA S	—	—	—	—	0	0	0	2	0	0	***31	0	0	—	—	—	—	—	0	**3	0	—	36
MA P	—	—	—	—	0	—	—	9	0	***18	0	***5	***35	—	—	—	—	1	5	3	0	—	76
FRA	—	—	—	0	0	0	0	0	0	0	**4	0	1	—	—	—	0	0	0	0	0	0	5
MT180° + RT	—	—	—	—	—	—	—	0	0	—	—	0	0	*5	***17	***15	0	0	0	2	0	—	39
PR 180°	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	1	*1	*2	1	0	0	5
PR + 180°	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	***9	0	***7	*1	0	—	17
PR − 180°	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	4	1	***6	***4	0	—	15
MT 0° + Pyg 0°	—	—	—	—	—	—	—	0	0	—	**21	0	3	—	—	—	0	—	0	1	0	—	25
ML MetaL	—	—	—	—	—	—	—	0	0	—	—	0	—	—	—	—	1	0	2	0	0	0	3
FPA MetaL	—	—	—	—	—	—	—	0	0	0	*4-z	0	0	—	—	—	***10	0	0	***8	0	0	22
Total	0	22	7	22	7	174	105	89	3	36	76	5	39	5	17	15	25	3	22	26	3	0	—

Tables 5 and 6 describe the codes for the behaviors listed below. Transitions with asterisks are significantly different from expected (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Transitions indicated with -z occur less frequently than expected.

Table 9. Behavioral transition matrix for summed frequency of transitions leading to a copulation of experienced *M. histrionica* adults ($N = 57$)

Preceding behavior	Succeeding behavior																			
	FAMf	MAFf	MAFp	MA + Pyg	MA + Pyg P	MA A	FA A	FN	MN	MA S	MA P	FRA	MT180° + RT	PR 180°	MT 0° + Pyg 0°	ML MetaL	FPA MetaL	FE	ME	PC	Total
NC	13	23	21	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	57
FAMf	0	—	—	0	—	0	***13	0	0	—	—	0	—	—	—	—	—	0	0	—	13
MAFf	—	0	—	***23	—	0	0	0	0	0	—	0	—	—	—	—	—	0	0	—	23
MAFp	—	—	0	—	***21	—	—	0	0	0	0	0	0	—	—	0	0	0	0	—	21
MA + Pyg	—	—	—	0	—	0	***36	0	0	0	—	0	—	—	—	—	—	0	0	—	36
MA + Pyg P	—	—	—	—	0	—	—	***9	0	***4	8	0	0	—	0	0	0	0	0	—	21
MA A	—	—	—	0	—	0	*154	24	0	*33	—	0	—	—	—	—	—	0	0	—	211
FA A	—	—	—	13	—	***185	0	0	**14	***5-z	—	0	—	—	—	—	—	0	0	—	217
FN	—	—	—	0	0	***24	0	0	0	0	***44	0	0	—	0	0	0	0	0	0	68
MN	—	—	—	0	0	0	***14	0	0	0	0	0	0	—	0	0	0	0	0	0	14
MA S	—	—	—	—	—	2	0	0	0	0	***67	0	0	—	—	—	0	0	0	—	69
MA P	—	—	—	—	—	0	0	***35	0	***27	0	***45	***92	—	—	—	0	0	0	—	199
FRA	—	—	—	0	0	0	0	0	0	0	*6-z	0	***39	—	0	0	0	0	0	0	45
MT180° + RT	—	—	—	—	—	—	—	0	0	—	—	0	0	***137	0	0	0	0	0	—	137
PR 180°	—	—	—	—	—	—	—	0	0	—	—	0	0	0	23	***18	***57	0	0	*39	137
MT 0° + Pyg 0°	—	—	—	—	—	—	—	0	0	—	***74	0	**6-z	—	0	—	0	0	0	—	80
ML MetaL	—	—	—	—	—	—	—	0	0	—	—	0	—	—	*3-z	0	2	0	0	**18	23
FPA MetaL	—	—	—	—	—	—	—	0	0	0	0	0	0	—	***54	***5	0	0	0	0	59
Total	13	23	21	36	21	211	217	68	14	69	199	45	137	137	80	23	59	0	0	57	—

Tables 5 and 6 describe the codes for the behaviors listed below. Transitions with asterisks are significantly different from expected (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Transitions indicated with -z occur less frequently than expected.

Table 10. Behavioral transition matrix for summed frequency of transitions leading to a missed copulation of experienced *M. histrionica* adults (*N* = 13)

Preceding behavior	Succeeding behavior																			
	FAMf	MAFf	MAFp	MA + Pyg	MA + Pyg P	MA A	FA A	FN	MN	MA S	MA P	FRA	MT180° + RT	PR 180°	MT 0° + Pyg 0°	ML MetaL	FPA MetaL	FE	ME	PC	Total
NC	0	**10	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	13
FAMf	0	—	—	0	—	0	0	0	0	—	—	0	—	—	—	—	—	0	0	—	0
MAFf	—	0	—	***10	—	0	0	0	0	0	—	0	—	—	—	—	—	0	0	—	10
MAFp	—	—	0	—	***3	—	—	0	0	0	0	0	0	—	—	0	0	0	0	—	3
MA + Pyg	—	—	—	0	—	0	***10	0	0	0	—	0	—	—	—	—	—	0	0	—	10
MA + Pyg P	—	—	—	—	0	—	—	1	0	1	1	0	0	—	0	0	0	0	0	—	3
MA A	—	—	—	0	—	0	31	***28	0	*6	—	0	—	—	—	—	—	4	0	—	69
FA A	—	—	—	0	—	***41	0	0	0	0	—	0	—	—	—	—	—	0	0	—	41
FN	—	—	—	0	0	***28	0	0	0	0	2	0	0	—	0	0	0	0	0	0	30
MN	—	—	—	0	0	0	0	0	0	0	0	0	0	—	0	0	0	0	0	0	0
MA S	—	—	—	—	—	0	0	0	0	0	***7	0	0	—	—	—	0	1	0	—	8
MA P	—	—	—	—	—	0	0	1	0	1	0	***3	***7	—	—	—	0	*2	1	—	15
FRA	—	—	—	0	0	0	0	0	0	0	**3	0	0	—	0	0	0	0	0	0	3
MT180° + RT	—	—	—	—	—	—	—	0	0	—	—	0	0	**7	0	0	0	0	0	—	7
PR 180°	—	—	—	—	—	—	—	0	0	—	—	0	0	0	1	0	**2	*2	***2	0	7
MT 0° + Pyg 0°	—	—	—	—	—	—	—	0	0	—	2	0	0	—	0	—	0	0	0	—	2
ML MetaL	—	—	—	—	—	—	—	0	0	—	—	0	—	—	0	0	0	0	0	0	0
FPA MetaL	—	—	—	—	—	—	—	0	0	0	0	0	0	—	1	0	0	1	0	0	2
Total	0	10	3	10	3	69	41	30	0	8	15	3	7	7	2	0	2	10	3	0	—

Tables 5 and 6 describe the codes for the behaviors listed below. Transitions with asterisks are significantly different from expected (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Transitions indicated with -*z* occur less frequently than expected.

fluctuation in body weight, which occurred in relatively small cages with florets of broccoli being the only other material present, presumably is due to the rapid intake and excretion of large volumes of fluids from the host plant material. Unlike other pentatomids such as *Chlorochroa* and *Thyanta* spp., which mostly feed on seeds rich in fats, oils, and proteins (Hall and Teetes 1982, Scudder and Thomas 1987, Zalom et al. 1997, Holtz 2002), *M. histrionica* feeds on fleshy plants with a high water content and in which nutrients are more dilute. Thus, *M. histrionica* may have to imbibe and excrete large volumes of relatively dilute and nutrient poor plant juices to obtain sufficient nutrition to survive and reproduce, resulting in the fluctuations in body weights that we observed. Furthermore, bugs only courted and mated in the presence of host material, so it was not possible to repeat the experiments in the absence of food as a method of reducing the background variation in body weights due to food intake and excretion.

Our results showed that naïve male *M. histrionica* took significantly longer to court and copulate with females than experienced males. More detailed examination, using all possible pairings of naïve and previously mated insects of both sexes, suggested that the previous experience of males influenced the duration of these behaviors, because the courtship and copulation times of naïve males with either naïve or experienced females were equivalent, and the courtship and copulation times of experienced males with either naïve or experienced females were also equivalent, but significantly shorter than the analogous periods for naïve males. If the durations of these behaviors were controlled by females, then it might be expected that the durations of courtship and copulation of naïve females with either naïve or experienced males should be equivalent, but this was clearly not the case. Thus, the more rapid performance of these behaviors by experienced males may reflect a learned behavior, or underlying changes in physiology associated with the first copulation. However, we cannot exclude an alternative possibility, that females might control the duration of copulation based on the amount or quality of materials that the male transfers during mating, assuming that there are differences in the ejaculate produced by virgin and previously mated males.

Naïve males did not show any preference for either naïve or experienced females. In contrast, experienced males mated more with unmated females than with previously mated females, suggesting that males were able to determine the mating status of females, and that they discriminate against previously mated females with which their assurance of paternity would be decreased. Alternatively, these results might also reflect experienced females discriminating against experienced males. The cues that one or both sexes might use to determine the mating status of potential partners are not known, but might include marking of females by males, or other changes to the cuticular chemistry that could be readily determined during courtship.

The close-range courtship of phytophagous stink bugs follows a characteristic series of steps, with all species examined to date exhibiting similar behaviors. The main behavioral steps include 1) a male approaching a female by walking, 2) antennation and headbutting of the female by the male, 3) abdominal elevation by receptive females, 4) male antennal and aedeagal stimulation of female abdomens, and 5) end-to-end copulation (Fish and Alcock 1973, Borges et al. 1987, Wang and Millar 1997). However, in *M. histrionica*, antennation of the female's abdomen by a male was often followed by the female raising her abdomen above the mid-axis of her body, and males did not attempt to raise female abdomens with their heads (the head-butting behavior referred to by other authors).

Volatile sex or aggregation pheromones seem to play a role in long-range attraction of females to males in phytophagous stink bugs, but these chemicals are not the only signals mediating mate location, because most phytophagous stink bugs are not strongly attracted into proximity to pheromone sources (Aldrich et al. 1987, 1991; Borges et al. 1987; James et al. 1994; McBrien et al. 2001). Instead, over shorter distances phytophagous stink bugs, including *M. histrionica*, use substrate-borne vibrational signals for sexual communication (Ota and Èokl 1991; Ryan and Walter 1992; Miklas et al. 2001, 2003a,b; Èokl et al. 2004), with the pheromone apparently stimulating females to produce vibrational signals that males can use to locate them once both sexes are on the same substrate (Miklas et al. 2003a). Other cues, such as visual signals or cues associated with the host plants, may act in concert with insect-produced signals, but they have not been investigated.

The courtship behavior transitional analyses illustrated several overall trends in the courtship behavior of *M. histrionica.* In all sequences, it was possible to divide the sequences into two phases regardless of sexual experience. The first phase was initiated when bugs were facing each other and cycling through the same behaviors repeatedly before moving out of the sequence, such as antennation of one another for prolonged periods followed by the female remaining motionless while the male continued to antennate her. The first phase ended with the male at the posterior of the female. The second phase then commenced, with the bugs cycling through another series of behaviors consisting of the male antennating the female posterior, the male turning 180° away from the female posterior while simultaneously rotating his pygophore, the pygophore rotating 180°, the male locating the female with his metathoracic legs, the female pushing the male away with her metathoracic legs, and the male turning to the head-to-tail position again while simultaneously turning the pygophore to its normal position. This second cycle then repeated until the male coupled with the female, or the male lost contact with the female.

The first phase seemed to be a female acceptance phase, in which the male antennated the female and the female antennated the male's antennae or re-

mained passive. For naïve bugs, the binomial tests of transition frequencies (Tables 7 and 8) and the transition probabilities (Fig. 2A and B) showed that after males antennated females' antennae, naïve females that did not copulate at the end of the sequence remained immobile proportionally more than females that did copulate and which exhibited the behavior of antennating males' antennae proportionally more than noncopulating females. The identical situation is evident for experienced bugs (Tables 9 and 10; Fig. 2C and D).

In contrast, in the second phase it seemed that males must perform a series of behaviors properly for copulation to ensue. The binomial tests of transition frequencies suggested that when in the second phase, the rotation of the pygophore by greater or <180° by naïve males and the resulting failure to couple the genitalia resulted in the female escaping directly or females rejecting males before escaping, whereas most of the sequences terminating with copulation in naïve bugs occurred from males persisting in courtship, returning to the beginning of the second phase and cycling through the sequence again until they successfully coupled with females. Experienced males also cycled through the second phase, with the sequence either ending in copulation or with one of the bugs moving away. Similarly, experienced bugs most commonly copulated directly after the male rotated the pygophore 180°, and subsequently located the female with his metathoracic legs, with the pygophore in the correct position for coupling the genitalia.

Failed courtships took place in both phases of the courtship. During phase 1, courtship failed most often at the step of the male antennating the female's antennae. If the female was not receptive, she simply moved away, and the sequence terminated. During the second phase, naïve females commonly escaped from males when the pygophore was not rotated correctly so that the genitalia could not couple, and the female pushed the male away with her metathoracic legs. Copulation failure for experienced males that passed phase 1 and which always rotated the pygophore correctly resulted from the female pushing the male away and escaping, or from the bugs moving away from each other. Whereas it is plausible that an unreceptive female would move away, it is not clear why the male would break off courtship and abandon the female after expending effort on the first stages of courtship, and on cycling through the second phase one or more times.

In summary, *M. histrionica* had a sexual maturation period of ≈1 wk under our rearing conditions. This sexual maturation period must be considered when using bugs in experiments related to reproductive behavior, such as searching for pheromones that might be used as signals in intersexual interactions. Most reproductive activity occurred ≈5 h after the onset of the photophase, providing a clear indication as to the optimal time to perform bioassays. The mating behaviors observed corresponded in general with reports from other species, but were more complex than previously noted. The pygophore rotation behavior, which seemed to be critically important for successful coupling, has been previously reported among bug species (e.g., Weber 1930).

Within the Heteroptera, prolonged mating has usually been described as mate-guarding to reduce sperm displacement upon reinsemination of the female (McLain 1980, Sillén-Tullberg 1981, McLain 1989, Carroll and Loye 1990, Carroll 1991, Alcock 1994, Hosokawa and Suzuki 2001). However, that only the first copulation is prolonged, and then only for naïve *M. histrionica* males, suggests other possible explanations. For example, unmated males may transfer more sperm and associated nutrients to females than previously mated males, as has been shown with other pentatomid species (e.g., Wang and Millar 1997). However, because the weights of *M. histrionica* of both sexes varied widely even in the absence of mating, the determination of whether males transfer substantial amounts of materials to females during copulation will have to be determined indirectly by methods other than measuring weight changes.

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