

Inter- and Intrasexual Interactions in *Anoplophora glabripennis* (Coleoptera: Cerambycidae) and the Impact of Different Sex Ratios

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Abstract

The behaviors of *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) during inter- and intrasexual interactions between both solitary and paired individuals at different sex ratios were observed on *Acer platanoides* L. (Sapindales: Sapindaceae) branches in the laboratory. Intrasexual contact was generally ignored by females, but between males resulted in battles from which one male ultimately retreated. When male size differed, larger males won battles, whereas equally sized males split the wins and battled longer. When males initiated intersexual contact, they quickly found and mounted the female. The female apparently determined if and how long the male could copulate with her by controlling access to her genital opening and males would eventually dismount if the female remained unreceptive. After successful copulation, males ignored female intruders and fought off male challengers, generally without dismounting. Some males dismounted females without attempting to copulate. Individual age and size were not predictive of either female or male choice in a mate. Mating duration was affected by both female receptivity and sex ratios present. Male interruptions of the pair shortened time in copula. Male-skewed sex ratios (1F:2M) significantly shortened the time a male would stay with an unreceptive female. Female-skewed sex ratios (2F:1M) did not impact mating duration. Probability of dispersal both from and within the branch was greater for smaller individuals of both sexes and for males when sex ratios were male-skewed. This information demonstrates how reproductive activity might be impacted as population densities decline during efforts at eradication of this species in North America.

Key words: Asian longhorned beetle, invasive species, copulation, oviposition, reproduction

Cerambycids employ different mating strategies that have evolved in response to the spatial distribution and abundance of the host resources required for females to produce progeny, as well as the ability of males to monopolize either the host resource or available females and successfully mate (Thornhill and Aklcock 1983, Hanks and Wang 2017). Some male Lamiinae (Coleoptera: Cerambycidae) are thought to defend territories that contain limited resources, while others defend females because the resources are too large or widespread to guard (Hanks and Wang 2017). In either strategy, intrasexual aggression between males occurs when they come into contact, and male reproductive success is directly tied to the number of females a male can find, defend, and mate. Male body size has been shown to play a role in the outcome of male competition, with larger males generally winning battles (Goldsmith et al. 1996, Togashi 1998, Kobayashi et al. 2003). Female intrasexual interactions in cerambycids are generally not aggressive (Hanks et al. 1996, Wang et al. 1996).

In addition to male competition, sexual selection in cerambycids also involves intersexual interactions that impact mating success. For

multiply mating species, factors that affect mating duration (time for both copulating and mate guarding) could alter male reproductive success since mating duration likely ties directly to the number of sperm transferred to and/or removed from female storage organs (Yokoi 1990, Alcock 1994, Simmons 2001). Factors shown to increase mating duration in cerambycids include increased female receptivity, decreased male density, larger female size, and earlier time of day (Droney and Thaker 2006, Keena and Sánchez 2018). Female receptivity, both pre- and post-copulation, can have a significant impact on mating duration and success. In many species of cerambycids, females are generally refractory; avoiding males, discouraging mounting, or preventing access to their genital opening once mounted (Michelsen 1963, 1966). Males respond to unreceptive females in many ways, including preventing escape, biting, palpating the female's elytra, and antennal wagging, but they will ultimately dismount if they cannot successfully copulate (Hanks and Wang 2017). Females may become unreceptive after initial copulations and may even attempt to push the male off her back, thus in part determining the mating duration (Keena and Sánchez 2018).

Sex ratio of conspecifics in the immediate environment can also have an impact on mating duration. With male-biased sex ratios, males guard mates longer to protect the female from rivals and transfer more sperm, but with female-biased sex ratios males guard mates less, enabling them to copulate with more females (Alcock 1994). However, greater likelihood of interference and harassment from rival males when sex ratios are male biased has been shown to reduce mating duration in the cerambycid *Tetraopes tetrophthalmus* (Forster) (Coleoptera: Cerambycidae) (Droney and Thaker 2006). Thus, there may be a trade-off between risk of injury in fighting off other males and the benefits of protecting the investment already made in the female. Female receptivity and choosiness may also vary with sex ratio and local density of conspecifics. Male rivalry in high density situations might promote mating by larger males (as these would win battles with smaller males) and provide the opportunity to mate with larger females (more fecund). Additionally, females may respond by being less discriminating (McLain and Boromisa 1987).

Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae) is a diurnal species in the subfamily Lamiinae. Adults find and mate with each other on their larval host trees. This beetle is a pest of healthy broadleaf trees both in its native range and where it has been introduced in North America and Europe (Yan and Qin 1992, Haack et al. 2010, Meng et al. 2015). Eradication efforts are underway for introduced populations in the United States and Europe because of the potential economic impact from injury or death of healthy trees within its preferred host genera (*Acer* [Aceraceae], *Populus* [Salicaceae], *Salix* [Salicaceae], and *Ulmus* [Ulmaceae]) (Meng et al. 2015). Male *A. glabripennis* actively seek females by palpating the tree surface to follow a sex trail pheromone the female deposits as she walks on the tree (Hoover et al. 2014, Graves et al. 2016). Virgin females also can locate males by following the volatile pheromone they produce when it is in combination with plant volatiles (Zhang et al. 2002; Nehme et al. 2009, 2010, 2014; Meng et al. 2014). Males quickly mount females after contacting them with their antennae and copulate with them in a series of 3–4 bouts (3–5 copulations each) during which the female chews oviposition pits (Zhang et al. 2003, Keena and Sánchez 2018). The males mate-guard between bouts while the female lays eggs that become fertilized within 43 min of the first copulation (Keena and Sánchez 2018). There is some evidence of assortative mating in this species, and females often reject males both pre- and postcopulation (Lingafelter and Hoebeke 2002, Keena and Sánchez 2018). When males encounter each other, they bite, kick, and strike each other with their antennae. A previous study has detailed the reproductive behaviors of isolated pairs, documented some differences between geographic populations, and shown that beetle age at mating affects female fecundity (Keena and Sánchez 2018).

This study sought to elucidate the population dynamics of *A. glabripennis* by examining behaviors during inter- and intrasexual interactions by both solitary and paired individuals at different sex ratios. We 1) documented the behavioral sequence following contact between individuals, 2) determined whether body size plays a role in the outcome of interactions, 3) determined how inter- and intrasexual interactions affect mating duration and taking flight, and 4) determined the impact of different local sex ratios on mating duration and success. This information will help predict what will happen as population densities decline during eradication efforts.

Materials and Methods

Insects and Rearing Conditions

The individuals used in the behavioral assays came from a colony that was founded with adults that emerged from infested branch

sections obtained in February 1999 from the Ravenswood, Chicago, IL, infestation (1,450 adults; 041.58°N, 087.42°W). Infested branch sections were transported under permit to the USDA-Forest Service quarantine facility in Ansonia, CT. Voucher specimens of each population were deposited at the Entomology Division of the Yale Peabody Museum of Natural History, New Haven, CT.

Observations were conducted in 2004 and 2005 using adults from the seventh generation of the Chicago population. This was the first generation when sufficient beetles were available to conduct this experiment. General colony-rearing methods used to obtain adults are given in Keena (2005) (using the artificial diet labeled AG2). Adults were held, both before and after the behavioral assays, at $25 \pm 2^\circ\text{C}$, $60 \pm 5\%$ RH, and a photoperiod of 16:8 (L:D) h. Newly emerged adults were held 4–5 d in 50-ml tubes (with a hole in the lid) in the dark to allow their exoskeleton to sclerotize before being weighed (digital scale to nearest 0.1 mg) and fed. Adults of both sexes were provided fresh *Acer platanoides* L. (Sapindales: Sapindaceae) (Norway maple) twigs (3–7 mm diameter with leaves removed) and held individually in 950-ml glass jars to prevent contact until bioassayed. Any females that copulated during the behavioral bioassays were held individually for 3–5 wk in a 3.8-liter glass jar and once per week provided with fresh *A. platanoides* twigs for food and an *A. platanoides* bolt (3–7 cm diameter and 20 cm long) with waxed ends as an oviposition substrate. Fresh *A. platanoides* twigs and bolts were obtained biweekly and monthly, respectively, and stored at 10°C and $\geq 80\%$ RH until used. Oviposition bolts were removed weekly (and replaced) from the jars containing mated females and held at 25°C for 4–5 d; then eggs were removed from under the bark and placed individually in the wells of a labeled 24-well tissue culture plate. The well plates were placed on a grate over water in a box to maintain $\sim 100\%$ RH, and the box was held in a chamber at 25°C until the eggs hatched. Females that copulated were held for 3–4 wk, and the proportion of eggs that hatched was the measurement of fertilization rate.

Observation of Behavioral Interactions

To evaluate interactions between individuals of the same sex, opposite sex, and between paired beetles and a third individual, 15 observations (three beetles in each observation) in four treatments were made (total of 60 observations). All beetles were between the ages of 11 and 41 d (average 20 d) with the exception of one 6-d-old female that was used inadvertently. To observe interactions, the beetles were placed on a 1.2-m-tall multibranched *A. platanoides* stem (3.5 cm diameter at the base) with all leaves removed. The stem was held upright with its base immersed in water and replaced with a new stem at least every 2 wk. The base of the stem was surrounded by a conical surface covered in fine-grain, black sandpaper to allow beetles to walk to the stem. Treatments involved introducing one beetle, letting it settle (stop walking for 30 s), and then adding a second beetle to allow it to interact with the first beetle before a third beetle was added. Each beetle was moved using a twig and placed at the base of the stem section facing upwards. The third beetle was added once the first two beetles had interacted; forming a pair-bond if of the opposite sex or contact with each other at least twice if of the same sex. A pair of individuals was considered to be ‘bonded’ if the male was mounted on the female and either actively copulating or remaining clasped to the female. The available beetles were randomly arranged in rows and sequentially used only avoiding reuse in the same treatment on the same day. The sequence of the sexes of the three beetles introduced in the four treatments were as follows: 1) male (M), M, female (F); 2) F, M, M; 3) F, M, F; and 4) F, F, M. When the first two beetles were of opposite sexes the female was

always introduced first since the males follow a female-released trail pheromone to find them on the host. Individuals were identified and differentiated within on the steam section using the beetle's size, elytral spot pattern, and other physical characteristics. In the absence of sufficient distinguishing characteristics, one elytral spot was colored red with a Sharpie (Newell Brands, Hoboken, NJ) pen. Room temperature was maintained at approximately 24°C and lighting was both from overhead fluorescent bulbs and natural light through a window. Observations began between 8:30 and 11:15 a.m. or 1:00 and 2:30 p.m. and averaged 60 min in length, with approximately half of trials for each treatment conducted in either morning or afternoon. Two to five observations (minimum of 5 min between) were made on any one day and were conducted as beetles were available from December 14 through February 25. Observations were continued until 1) a pair-bond had formed if the first two beetles were of the same sex, 2) the third beetle had contacted the pair at least twice, or 3) the original pair separated. Observations were ended if no pair-bond formed after at least 3 male–female contacts and 60 min had elapsed or if males continued to bite each other for more than 15 min.

Only a limited number of beetles were available (53 males and 46 females), so it was necessary to reuse at least some beetles in subsequent trials. However, females observed copulating for 3 min, with full aedeagus extension, were removed from the experimental pool to determine egg fertility. This timeframe for considering the copulation successful was based on the information found in Keena and Sánchez (2018). In addition, when a male inserted his aedeagus but did not achieve the full extension, it was considered a copulation attempt that was not successful in part of the analyses. The same individuals were never used more than three times in any single treatment, and at least a single day elapsed between trials with the same individuals.

Thirty-eight different kinds of behaviors were recorded during observations (Keena and Sánchez 2018; Supp Table 1 online only), but only a subset of behaviors pertinent to this study are reported and discussed. Each individual incident of contact or mounting, segregated by the combination of the sexes of the actor and recipient (male-on-female, male-on-male, and female-on-female), was the unit of replication. Data on each mounting type, duration and winners of battles, length of time in copula, and flight incidents were also recorded. The winner of battles was the beetle that remained in the location where the interaction occurred while the loser quickly moved away. A female was considered to be receptive if she allowed access to her genital opening and was not exhibiting rejection behaviors as described in Keena and Sánchez (2018). All observations were recorded in behavioral analysis software, The Observer XT 11.5 (Noldus Information Technology 2013).

To evaluate relative male weights of winners and losers in battles, males were classified as similar in size if the difference in body mass was $\leq 10\%$ of the body mass and larger or smaller if the mass difference was $>10\%$. Ten percent was chosen since male size (which is correlated with body mass) differences of 7–8% have been shown to affect mating success and behaviors in other cerambycids (Hanks et al. 1996, Fukaya et al. 2004).

Statistical Analysis

The fit of the data for each parameter to various distributions was first evaluated using PROC UNIVARIATE (SAS Institute 2015). The Shapiro–Wilk test was used to assess normality. All continuous variables were analyzed in PROC GLIMMIX (SAS Institute 2015) using a completely randomized design and a gamma distribution with a log link for all but the time from female contact to male

mounting which required a lognormal distribution with an identity link. Treatment was used as the fixed effect for time from contact to mounting. Potential predictors of female receptivity and the probability of beetle flight, two dichotomous variables, were evaluated using a binary distribution with a logit link. Male age and body mass (mg) were evaluated as predictors of female receptivity. The fixed effect of female present or absent on the steam section was used for analysis of duration of male-on-male mounts. The fixed effects of female present or absent, relative size of the male winner (smaller, equal, or larger than the loser), and the interaction between the two were used for duration of male battles. Sex ratio, sex, and the interaction of the two were evaluated as predictors of the probability of the individual flying during the observation. Interruption type (female on pair, male on pair, or no interruption) was used as a fixed effect for time in copula, number of copulation attempts, and combined with success of the copulation (length of the full copulations achieved) for the duration of the pair-bond. For each model, residuals were evaluated using Levene's test to assess homogeneity of variance. Differences among means were determined by the least-squares means test with $\alpha = 0.05$ and a conservative Tukey–Kramer grouping (SAS Institute 2015). PROC NLIN and MODEL (SAS Institute 2015) were used to determine whether there was a linear relationship between male or female body mass and total time a pair spent in copula. All chi-square tests were conducted using Statistix 10 software (Statistix 2013).

Results

Interactions Between Unpaired Individuals of the Opposite Sex

Single males that contacted females mounted them 83% of the time, either with or without additional contacts (Fig. 1). Eight percent of males that mounted then dismounted immediately and 23% of the males mounted on a receptive female dismounted in less than a minute without attempting to copulate. Three-quarters of these cases in which the male did not attempt to copulate were from the MMF treatment where the male had previously been involved in a battle with another male. Forty-six percent of mounted females were immediately receptive and another 13% became receptive after the male performed his antennal wag. Neither male body mass ($F = 0.0$; $df = 41, 39$; $P = 1.0$) nor male age ($F = 0.04$; $df = 18, 39$; $P = 1.0$) was significant predictor of female receptivity. Receptive females stopped moving, occasionally lifted their abdominal tip, and allowed access to their genital opening. Sixty-two percent of females with which males successfully copulated performed a 'rejection display' after a prolonged pair-bond (≥ 3 complete copulation bouts, each with multiple successful copulations, see Keena and Sánchez 2018 for more complete description). In the rejection display, females would exhibit one or more of the following behaviors: kicking male with hind legs, striking male with her antennae, making quick turns, walking with male mounted through spaces not large enough for both to fit (apparently to push him off her back). Ninety-five percent of males that unsuccessfully tried to copulate (female remained unreceptive) dismounted before the end of the trial. In addition, 34% of mounted males dismounted <1 min after the female began a rejection display following copulation, while the others persisted in mate guarding and wagging their antennae.

The time from male contact to mounting varied significantly by treatment ($F = 4.47$; $df = 3, 76$; $P = 0.0006$; Table 1). When two males had encounters with each other prior to female contact (MMF treatment), the time to mounting was significantly longer than when the female contact occurred with no prior interactions in treatment

Opposite Sex interactions

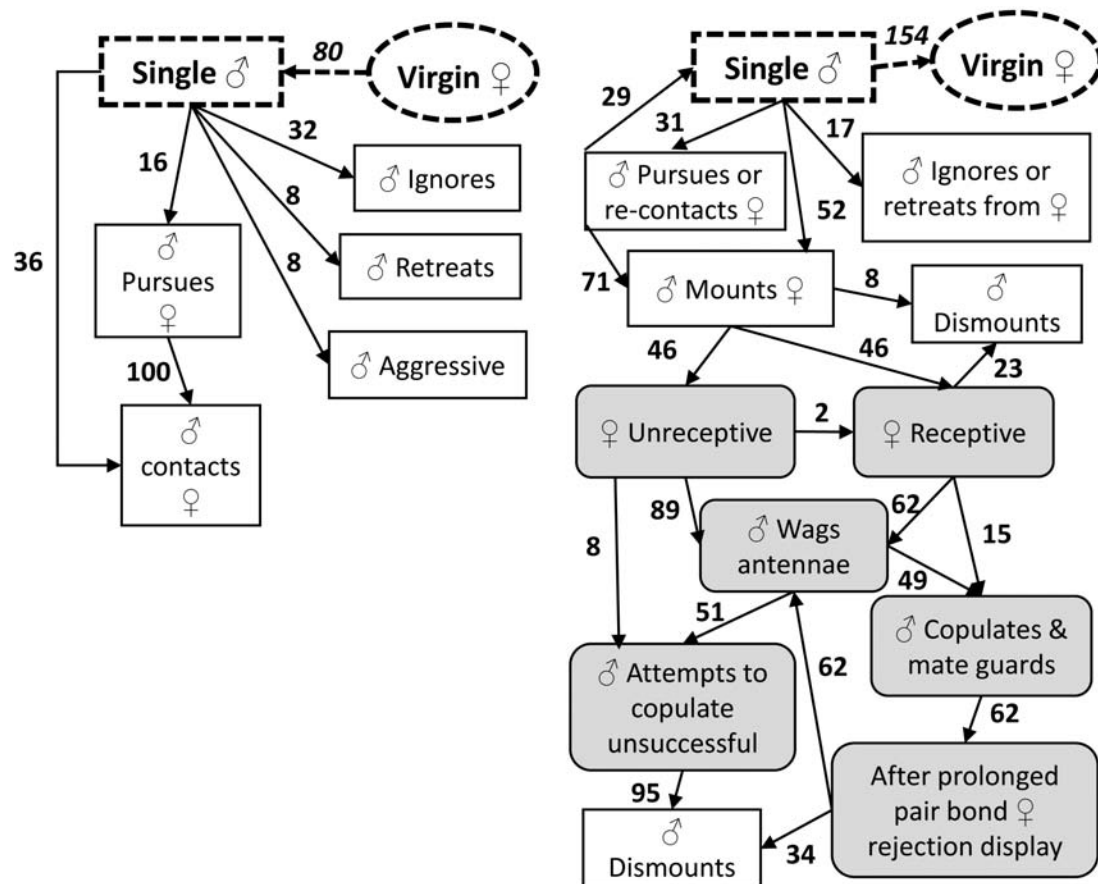


Fig. 1. Outline of behavioral sequences observed for opposite sex contacts during three-beetle interactions. Ovals are female actions, rectangles are male actions, and shaded boxes are actions of pair-bonded individuals. Dashed arrows and outlines indicate initial contact type, and the number is the total of that type of encounters. Values on the pathway sequence are the percentage of individuals that moved to the indicated next step out of those that entered the current step. These contact types could have occurred in any of the treatments.

Table 1. Time from initial contact until male mounted the female in *Anoplophora glabripennis*, mean $\pm$ SE (*n*)

Treatment	Male initially contacted female	Female initially contacted male
MMF	6.1 $\pm$ 0.8 (26)a	7.5 $\pm$ 1.3 (9)a
FFM	4.1 $\pm$ 0.7 (14)ab	7.1 $\pm$ 1.4 (6)a
FMM	3.8 $\pm$ 0.5 (25)ab	5.9 $\pm$ 1.4 (7)a
FMF	2.9 $\pm$ 0.5 (15)b	7.5 $\pm$ 1.3 (7)a

Means within the same column followed by the same letter were not significantly different ($P < 0.05$, Tukey–Kramer test). The sequence of the sexes of the three beetles introduced in the four treatments was as follows: 1) male (M), M, female (F); 2) F, M, M; 3) F, M, F; and 4) F, F, M.

FMF but not treatment FMM. Time from initial female contact of a male to his mounting her did not vary by treatment ($F = 0.12$; $df = 3$, 25; $P = 0.9475$; Table 1). Also, only 52% of males that were contacted by females either pursued the female or initiated additional contacts that could result in mounting (Fig. 1).

Interactions Between Unpaired Individuals of the Same Sex

When virgin females contacted each other, 63% ignored the contact (Fig. 2). If one female became aggressive after the contact by

either striking the other with antennae or kicking, 29% of the time the aggressor would mount the other female. Female-on-female mounts lasted an average ($\pm$ SE) of 160 ± 71 s. Ultimately, the females would separate and occasionally clean their antennae after the encounter.

Male–male contacts, either after initial or subsequent contacts, ultimately resulted in males battling if one male did not quickly retreat (Fig. 2). Male battles involved lashing with antennae, biting of antennae and legs, kicking, mounting, and head butting (Fig. 3B). The relative size of the males involved in the battle had a significant effect on the duration of the battle ($F = 11.82$; $df = 2$, 162; $P < 0.0001$). When males were of similar size (within 10%), the battle was longer than if they differed by a greater amount (Table 2). Relatively larger males won more battles (Yates' $\chi^2 = 9.56$, $P = 0.0020$) while similarly sized males split the wins (Yates' $\chi^2 = 0.36$, $P = 0.5466$, Fig. 4). Male-on-male mounts lasted an average ($\pm$ SE) of 141 ± 38 s and 10% of the time the mounting male attempted to copulate (in 75% of cases the male that was mounted had had prior contact with a female). The duration of male-on-male mounts did not differ significantly with female presence at the time of the mount ($F = 0.02$; $df = 1$, 52; $P = 0.9007$). However, if one male had prior contact (duration 76 ± 11 s, $n = 107$) with a female, the duration of the battle that followed was significantly longer ($F = 7.06$; $df = 1$, 163; $P = 0.0087$) than if neither had prior contact with a female (duration 39 ± 9 s, $n = 59$).

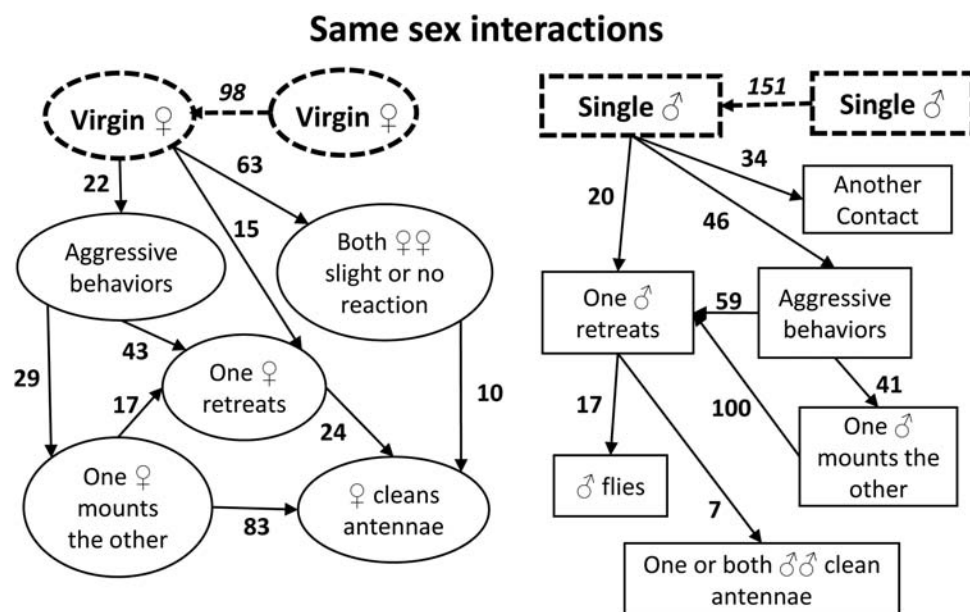


Fig. 2. Outline of behavioral sequences observed for same sex contacts that occurred during three-beetle interactions. Ovals are female actions, and rectangles are male actions. Dashed lines and dashed outlines of individuals represent initial contact type, and the number is the total of that type of encounters. Values on the pathway sequence are the percentage of individuals that moved to the indicated next step out of those that entered the current step. These contact types were only possible in certain treatments (e.g., male on male in treatments where two males were present).

Interactions Between a Pair and an Unpaired Individual

Pair-bonded individuals ignored (continued same behaviors) contact by a virgin female 80% of the time (Fig. 5). In other cases, some aggressive behavior occurred: pair-bonded individuals struck the invader female with their antennae, the invader female struck the pair with her antennae, or the pair-bonded female mounted the invader female (Fig. 3A), or the invader female mounted the pair. The pair-bonded male never dismounted following contact by the second female, and the invader female either retreated or stayed close by, often contacting the pair multiple times. The invader female also occasionally cleaned her antennae after contact. In only 3 out of the 30 observations with two females and one male did the male dismount the first female after she began rejection behaviors and mount the second female.

Fifty-one percent of the time one or both males exhibited aggressive behaviors after a single male contacted a pair and the pair-bonded male dismounted in 39% of those cases to battle the other male (Fig. 5). Males would also mount each other or both mount the female (Fig. 3C) during their battles over the female. The invader male retreated, either before or after aggressive behaviors, 44% of the time (59 out of 135 encounters). The pair-bonded male stayed mounted on the female 70% of the time (94 out of 135 encounters) and dismounted but returned to the female 7% of the time (10 out of 135 encounters). In 7% of the cases, the invader male ultimately found and mounted the female (10 out of 135 encounters), sometimes after he had initially retreated from the originally pair-bonded male. Males palpated the substrate presumably in searching for the female. Sixteen percent of the time the female retreated after being dismounted and neither male found her after the battle (21 out of 135 encounters).

The type of interruption (by a male or female; or no interruption) had a significant effect on the total time the pair spent in copula ($F = 6.72$, $df = 2$, 186 , $P = 0.0015$; Table 3). If a pair was interrupted by a female, the time in copula was the same as if no interruption

had occurred. However, if a male contacted a pair, the time in copula was significantly less than either no interruption or a female interruption. The percentage of copulatory attempts that were successful (resulted in full aedeagus extension) was not affected by interruption type ($F = 0.67$, $df = 2$, 77 , $P = 0.5145$; Table 3), nor was the average time the male remained mounted on the female if at least one copulation lasted ≥ 60 s (Table 4). However, when males were not able to establish a copulation ≥ 60 s, they dismounted sooner with no interruption or with a male interruption than males that had longer copulations, regardless of the interrupting sex. When the female was unreceptive, all copulation attempts lasted less than 60 s.

Both males (26 of 52) and females (23 of 46) flew during the observations. Females flew after same sex interactions, after an aggressive encounter with a pair, and after a male dismounted to interact with another male. Males flew after battles with other single males, aggressive interaction with a pair-bonded male, or occasionally after contact with a female when a male to male battle had previously occurred. Three individuals of each sex flew shortly after they were placed on the branch with no contact with another beetle. Sixty-two percent of the individuals that took flight flew again after being returned to the branch and making further contact with other beetles. The probability that a given individual beetle would take flight varied significantly depending on the sex of the individual and the sex ratio present ($F = 9.29$; $df = 1, 176$; $P = 0.003$; Fig. 6). When the sex ratio was male biased (1F:2M), the probability of males taking flight was higher than when the sex ratio was female biased (2F:1M). The probability of female flight did not differ with sex ratio and was not significantly different than that of males regardless of sex ratio.

Discussion

Male *A. glabripennis* both compete for territory when no female is present and tenaciously defend mates. When males battled, the larger male won and the smaller male retreated when no female was present. However, when a male had already pair-bonded with a female he

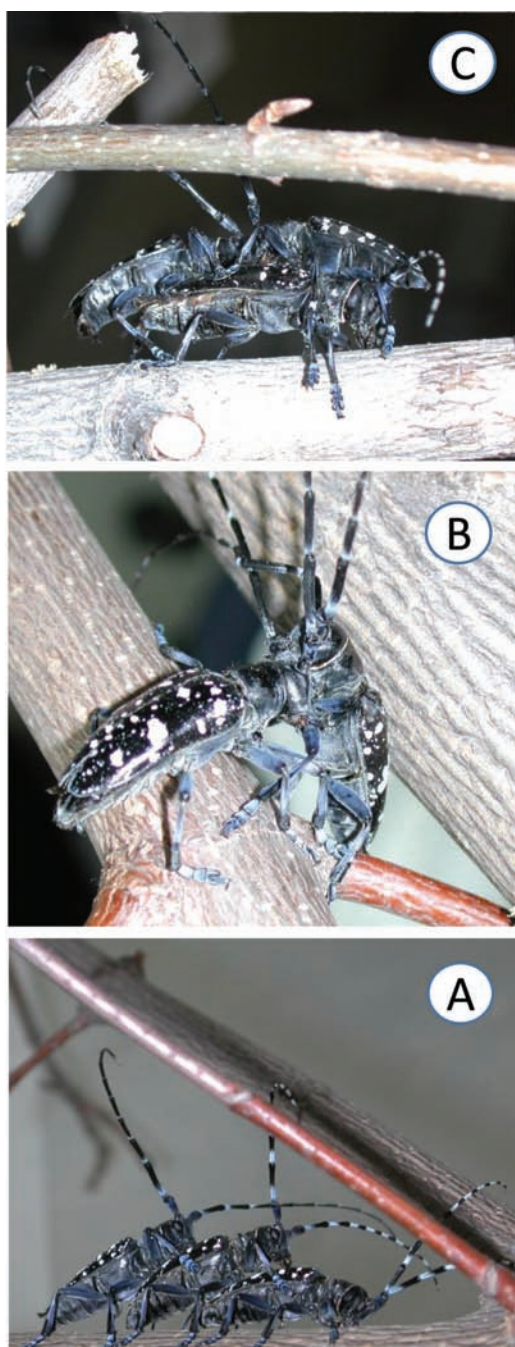


Fig. 3. *Anoplophora glabripennis*: (A) mating pair mounted on a female, (B) males battling, and (C) males battling on a female.

fought off other males usually without dismounting and the intruder rarely took possession of the female. The male completion behaviors observed in this study are consistent with what has been documented for *Monochamus* (Dejean) species and *T. tetraphthalmus* (Forster), including the male-on-smale mounting that is not seen in all cerambycids (Hughes 1979, McInnis and Boromisa 1987, Togashi 1998, Kobayashi et al. 2003, Droney and Thaker 2006). However, *A. glabripennis* male interactions were apparently more violent than those of its congener *Anoplophora chinensis* (Forster) (Coleoptera: Cerambycidae) (synonymous with *Anoplophora malasiaca*) whose males only stroke each other's antennae and then retreat (Wang et al. 1996). This may indicate that male competition for mates has a

Table 2. Effects of the size of male *Anoplophora glabripennis* that won battles on the duration of the battles, mean $\pm$ SE (n)

Winner size relative to loser	Duration of male battle (s)
Similar	95.7 $\pm$ 15.9 (86)a
Smaller	31.1 $\pm$ 20.8 (21)b
Larger	46.7 $\pm$ 13.8 (59)b

To evaluate relative male body mass of winners and losers in battles, males were classified as similar in size if the difference in mass was $\leq 10\%$ of the body weight and larger or smaller if the mass difference was $>10\%$. Means followed by the same letter were not significantly different ($P < 0.05$, Tukey-Kramer test).

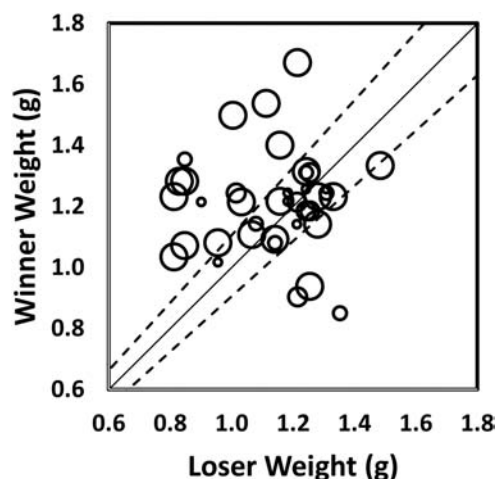


Fig. 4. Relationship between body masses of 'winning' and 'losing' males in battles between two males. The size of each circle represents the proportion of the interactions that the specific winner in the pair won (largest circles are 100% and smallest $\leq 20\%$); in some cases, both males won some of the battles that occurred between them so there are two separate points on the graph for that particular pair. The solid line represents equally sized males, and the area between the dashed lines represents $<5\%$ difference in size between males.

greater impact on male reproductive success in *A. glabripennis* than in *A. chinensis* despite similar mating behaviors and sharing one of the male produced volatile pheromone components.

Female to female contact, regardless of mating status, was rarely aggressive. Similar nonaggressive behaviors have been seen in other Lamiinae females, including *A. chinensis*, (McInnis and Boromisa 1987; Wang et al. 1990, 1996; Droney and Thaker 2006), but female on female or female on pair mountings have not been previously reported. There are examples from other animals (e.g., two-spotted goby, *Gobioculcus flavescens* F. [Perciformes: Gobiidae] and European lobster, *Homarus gammarus* L. [Decapoda: Nephropidae]) where females become aggressive only when the sex ratio is female biased (Weir et al. 2011). The aggressive female behaviors in *A. glabripennis* primarily occurred when the two females in the observation made multiple contacts with each other (either singly or when one was in a pair-bond) which could have been perceived by the beetles as a more female-biased sex ratio than was actually present (2F:1M).

Female *A. glabripennis* controlled access to their genital opening thus determining whether and when a male could copulate with them. As with many cerambycid species and previously seen in *A. glabripennis* (Keena and Sánchez 2018), only about half the younger virgin females are receptive when first mounted by a male, but in this study neither male body mass nor age was predictor

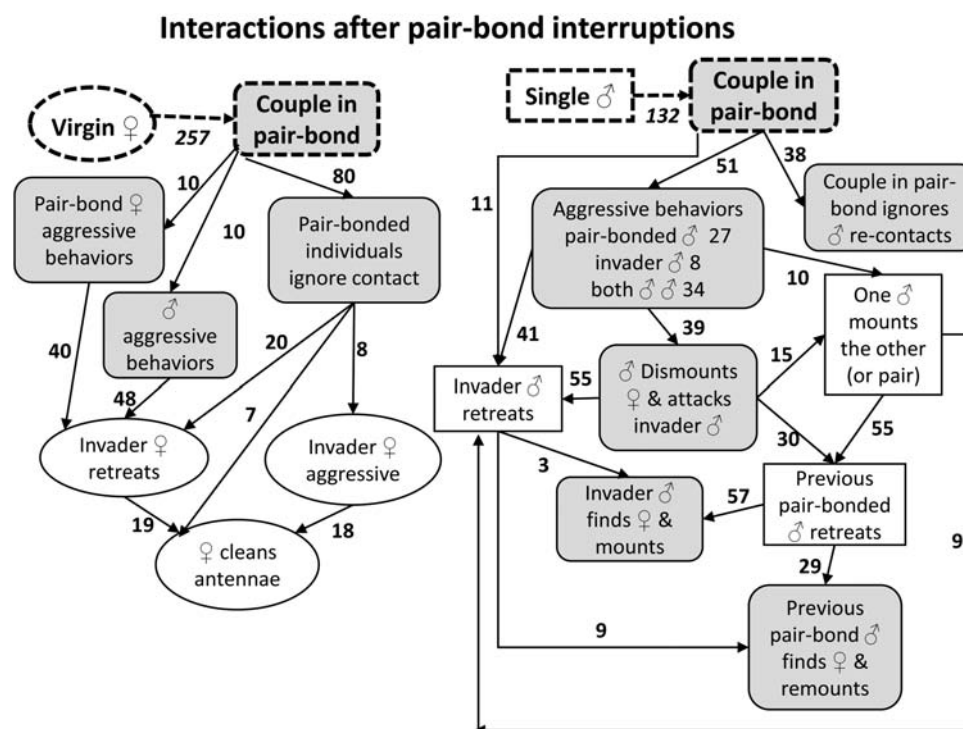


Fig. 5. Outline of behavioral sequences observed following contacts of pair-bonded individuals by a third beetle during three-beetle interactions. Ovals are female actions, rectangles are male actions, and shaded rectangles are actions of pair-bonded individuals. Dashed lines and dashed outlines of individuals or pairs represent initial contact type, and the number is the total of that type of encounters. Values on the pathway sequence are the percentage of individuals that moved to the indicated next step out of those that entered the current step. These contact types were only possible in certain treatments (e.g., male on pair in treatments where two males were present).

Table 3. Effects of interruption type on the average time mating *Anoplophora glabripennis* pairs spent in full copula and percent of copulatory attempts the male made where the aedeagus was fully extended, mean $\pm$ SE (n)

Type of interruption	Time in full copula (s)	Percentage of copulation attempts that were full
Female	112.8 $\pm$ 13.6 (44)b	41.6 $\pm$ 19.6 (21)a
Male	74.5 $\pm$ 7.0 (72)a	19.9 $\pm$ 9.0 (23)a
No interruption	117.3 $\pm$ 11.0 (73)b	25.2 $\pm$ 9.1 (36)a

Any copulation attempt either in progress or made after the male in the pair had made contact with the third beetle on the branch was considered to be interrupted (full aedeagus bridge formation to retraction). Means within the same column followed by the same letter are not significantly different from each other at $P < 0.05$ using the Tukey–Kramer post hoc test.

of female receptivity. This is not too surprising since there is little direct evidence that insect females actually discriminate between potential partners under natural conditions (Thornhill and Akloock 1983). These younger virgin females may simply not have mature eggs ready to be fertilized or have determined that the conditions are not suitable for reproduction. There is some variation in when *A. glabripennis* females initiate oviposition, and females prefer to lay eggs under the bark of branches that are of a larger diameter than were used in these observations (Keena 2002).

About 20% of males mounted females then dismounted soon after without attempting to copulate. It is possible that these males were rejecting those females for some reason. A preference for larger females has been seen in some other Lamiinae (McLain and Boromisa 1987, Droney and Thaker 2006), and it has been suggested that both sexes of *A. glabripennis* prefer strong active mates (Lingafelter and

Table 4. Effects of interruption type on length of time, mean $\pm$ SE (n) in min, male *Anoplophora glabripennis* remained mounted on a female (includes both copulation and mate guarding) broken down by type of full copulations (full aedeagus extension) that occurred

Interruption of mating pair	Mean duration of mount (min)	
	At least one full copulation lasting ≥ 60 s	All full copulations lasting < 60 s
Female	31.1 $\pm$ 7.3 (24)a	21.5 $\pm$ 6.1 (14)ab
Male	28.5 $\pm$ 7.4 (18)a	10.9 $\pm$ 2.2 (32)bc
No interruption	22.1 $\pm$ 9.2 (5)ab	5.2 $\pm$ 1.2 (21)c

Means within the table followed by the same letter are not significantly different from each other at $P < 0.05$ using the Tukey–Kramer post hoc test. Statistics: copulation type, $F = 21.81$, $df = 1, 107$, $P < 0.0001$; interruption type, $F = 3.93$, $df = 2, 16$, $P = 0.0405$; and interaction between copulation and interruption types, $F = 2.31$, $df = 2, 107$, $P = 0.1039$.

Hoebeker 2002). We did not specifically test for size assortative mating so further investigation would be needed to determine whether this was occurring. However, the majority of the males that exhibited this behavior had been involved in a battle with another male within the last 5 min. Maybe the battle triggers something in the male that reduces his ability to sense the female contact pheromone during these quick mount and dismounts.

This study's observations that a male could rapidly locate a female again on a multi-forked branch after dismounting to fight off an intruder led to the investigation and ultimate identification of a female produced trail sex pheromone in this species (Hoover et al. 2014).

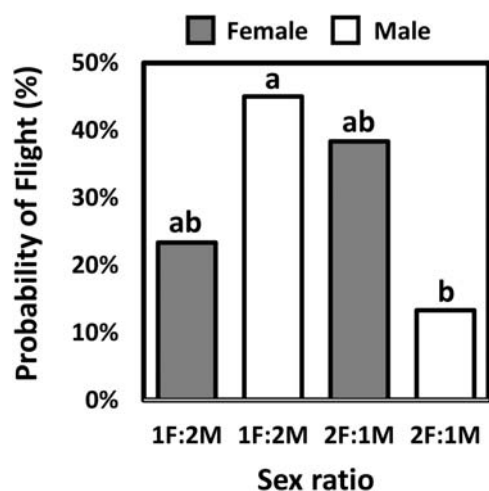


Fig. 6. Probability of *Anoplophora glabripennis* beetles taking flight during observation by sex and sex ratio. Bars with the same letter are not significantly different from each other at $P < 0.05$.

Since the branch was not changed after every observation in our study, residual trail pheromone from previous trials could have influenced the observed behaviors. However, we saw no evidence that males followed old trails, since they always appeared to track the females of the current observation. Second, although the duration of the biological activity of the trail pheromone on a branch has not been determined, the two major components have enthalpy of vaporization at standard condition values (66 kJ/mol, www.chemeo.com) that would indicate they dissipate fairly quickly. This would be expected since females move around quite a bit and so it would need to dissipate or males would waste energy following a trail that no longer leads to a mate. For example, the trail pheromones in ants dissipate quickly and need to be reinforced to be followed (Saran et al. 2007). More work would be needed to see whether trail pheromone persistence could affect perceived local densities of females even if it was not followed.

Mating duration was affected by the timing of female rejection displays after multiple copulations and the sex of the interrupting individual. After multiple copulations, the females became less receptive and began rejection displays as was previously documented for *A. glabripennis* pairings in the absence of additional adults (Keena and Sánchez 2018). Most males persisted for a time in mate guarding even after the female was not receptive but interruptions by competitors resulted in dismounting by the male. When the unreceptive female was dismounted, she quickly distanced herself (some flew away) from the males, especially if they were fighting. Female interruptions of a mating pair did not impact mating duration even if the second female was very persistent at contacting the pair. Further work to determine how many females a male can successfully copulate with and determine the effects of multiple male partners and copulations on female fertility may help explain the underlying selective pressures that drive these behaviors.

These findings suggest that even at low population densities (three beetles on a steam section) negative encounters between *A. glabripennis* individuals of the same sex will cause the beetles to spread out on the host (run or fly) and occasionally fly away off the host. When the sex ratio skews toward females, virgin females will leave the natal host seeking a mate (especially after negative intrasexual encounters as documented here) as trap catch in low-density populations has shown (Nehme et al. 2014). Similarly as sex ratios skew toward males, competition for mates will increase and males will distance themselves from each other after battles. This is consistent with field

studies that showed most beetle movement was within-tree, with only a small proportion of the population flew longer distances (Smith et al. 2004, Williams et al. 2004, Bancroft and Smith 2005).

These results have some management implications for the eradication programs. First, there is an indication that some *A. glabripennis* may fly away from a tree after negative conspecific interactions, even at low densities. Intrasexual encounters and resulting aggression would be expected to be greater when population densities are high and host quality is concomitantly declining resulting in increased dispersal, but dispersal in low-density populations is not often considered. A field study of the Laminiae *T. tetraphthalmus* has documented the greatest dispersal from areas of low population density (McLain and Boromisa 1987). If this low-density dispersal does occur in the natural environment, it may make finding the last infested trees more difficult, but could increase female trap catch and the number of beetles that never find mates, both of which help reduce overall population density as is desired in an eradication program. Secondly, there is no indication that female fertility will be affected by population density or sex ratio as long as females find a mate(s). At low population densities, single pairs that are not disrupted will stay together long enough for the female to remain fertile for life (Keena and Sánchez 2018). At higher population densities, 1 min of copulation is sufficient to transfer sperm, and the likelihood that a female will have multiple mates, again ensures she is fertile for life.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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