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Research Article

Reproductive Behaviors of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in the Laboratory

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Abstract

The reproductive behaviors of individual pairs of *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae)—all combinations of three populations and three different ages—were observed in glass jars in the laboratory on *Acer saccharum* Marshall (Sapindales: Sapindaceae) host material. The virgin female occasionally made first contact, but mounting did not occur until the male antennated or palpated the female. If the female was receptive (older females initially less receptive than younger ones), the male mated with her immediately after mounting and initiated a prolonged pair-bond. When the female was not receptive, some males abandoned the attempt while most performed a short antennal wagging behavior. During the pair-bond, the male continuously grasped the female's elytral margins with his prothoracic tarsi or both pro- and mesothoracic tarsi. The male copulated in a series of three to four bouts (averaging three to five copulations each) during which the female chewed oviposition sites or walked on the host. Between bouts, the female oviposited and fertile eggs were deposited as soon as 43 min after the first copulation. Females became unreceptive again after copulation and the duration of the pair-bond depended on the male's ability to remain mounted. Some population differences were seen which may be climatic adaptations. A single pair-bond was sufficient for the female to achieve ~60% fertility for her lifetime, but female fecundity declined with age at mating. Under eradication conditions, mates will become more difficult to find and females that find mates will likely produce fewer progeny because they will be older at the time of mating.

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

The reproductive behaviors of the subfamily Lamiinae of the cerambycids have been studied primarily for species of economic importance. The reproductive behavioral sequence has been studied, at least in part, in 16 species of Lamiinae (Coleoptera: Cerambycidae) (Supp Table S1 [online only]) including *Anoplophora chinensis* (Forster) (Coleoptera: Cerambycidae) (Wang et al. 1996a) and *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) (Zhou et al. 1984, Lingafelter and Hoebeke 2002, Morewood et al. 2004). Aspects of *A. glabripennis* reproductive biology have been investigated, including pheromones, oviposition, and daily patterns of activity (He and Huang 1993, Li and Liu 1997, Li et al. 1999, Keena 2002, Smith et al. 2002, Lance et al. 2003, Zhang et al. 2003, Morewood et al. 2004, Keena 2006, Hoover et al. 2014). However, the full reproductive behavioral sequence for *A. glabripennis* has not been described, geographic populations have not been compared to see whether there are differences between them, and the effects of various demographic factors (e.g., beetle age) on reproductive behaviors has not been assessed.

The reproductive behaviors of *A. glabripennis* are typical of diurnally active species of the subfamily Lamiinae. Newly emerged *A. glabripennis* adults can copulate and males do have mature sperm, but maturation feeding increases the probability of successful sperm transfer and is necessary for egg development (Li and Liu 1997). Males are more active than females and will actively search for a female by palpating the tree surface and following a sex trail pheromone laid down by the female as she walks across the surface (Hoover et al. 2014, Graves et al. 2016). Virgin females also can follow a male produced pheromone in combination with host volatiles to locate the males (Zhang et al. 2002; Nehme et al. 2009, 2010, 2014; Meng et al. 2014). Males quickly attempt to mount females and mate after sensing the contact pheromone on her body with their antennae or palps (Zhang et al. 2003). Visual cues appear to be important in male mate location; when the male's eyes are covered, he fails to find the female even after antennal contact (He and Huang 1993). Field observations in China report that mating peaks between 2 and 6 pm, prolonged pair-bonds (including mate guarding while mounted) last 1–2 hr, and individual copulation events last an average of 5–10 min (Zhou et al. 1984, Lingafelter and Hoebeke 2002). Both sexes repeatedly mate, when bonded with an individual and with multiple partners (Morewood et al. 2004). There is some

evidence that both sexes choose strong active mates and that females are more choosy than males (Lingafelter and Hoebeke 2002). Females chew pits in the bark and deposit single eggs at the cambial interface in some, but not all of the pits. Female fecundity increases with weight and varies depending on the quality and species of host from which she emerged (Keena 2002). Females oviposit more on *Acer* sp. than on *Salix* sp. (Smith et al. 2002), and oviposition strategies vary between populations (Keena 2002). Temperature also affects both fecundity and adult longevity; the optimum temperature for both is at or slightly above 20°C and both decline above and below that temperature (Keena 2006).

Anoplophora glabripennis is considered a major pest of several deciduous broadleaf tree species in its native range in China (Yan and Qin 1992). It is under eradication in both North America and Europe where it has been introduced via infested wood packaging material used in international trade (Haack et al. 2010, Meng et al. 2015). It attacks apparently healthy trees and although it has a broad host range, it prefers the genera *Acer*, *Populus*, *Salix*, and *Ulmus* (Meng et al. 2015). Larvae tunnel initially in the sapwood but enter the heartwood as they grow, then pupate in a chamber they chew, leaving about 1 cm for the adult to chew through when it emerges. The larval tunneling damages the vascular system of branches, weakens the structural integrity of the tree, and eventually kills it. In the northeastern United States alone, *A. glabripennis* has the potential to cause additional millions of dollars of loss in urban trees, timber and maple syrup production, and billions of dollars of lost tourism revenue in the fall (USDA-APHIS-MRP 2015). Because of the potential economic impacts, the cooperative eradication program is committed to locating and destroying all infested trees (USDA-APHIS-MRP 2015).

There is a critical need to better understand the reproductive behaviors of this beetle to provide a biological basis for predicting the population dynamics, especially as beetle populations significantly decline due to eradication efforts in the non-native habitats. Understanding the behaviors of isolated pairs, determining factors that affect fecundity and fertility, and determining how intra- and inter-sex interactions affect mating success are important aspects of the reproductive behaviors that can affect the population dynamics of this insect. Here we present the reproductive behavioral sequence of *A. glabripennis* obtained by observing 45 isolated pairs until natural separation or for up to 6 hr, whichever came first. We also assess the effects of geographic population source and beetle age at the time of mating on the timing, occurrence, and duration of specific events. Implications of the results for predicting population dynamics of *A. glabripennis* and improving monitoring and management/eradication methods are discussed.

Materials and Methods

Insects and Rearing Conditions

Individuals used in the behavioral assays were from colonies established using adults from infested branch sections obtained in 1999 from the Ravenswood, Chicago, IL, infestation (041.58°N and 087.42°W), and 1999 from the Bayside, Queens, NY infestation (040.45°N and 073.45°W) or larvae obtained from Hohhot City, Inner Mongolia, China, in 2001 (040.82°N and 111.60°E). The 6-hr single-pair observations were conducted in 2003 using adults from the fifth generation of the Chicago and Bayside populations and the second generation of the Hohhot City population. Laboratory rearing may have had some effect on the behaviors of this insect, although the geographic populations were reared under protocols to conserve genetic diversity. However, there are reported differences between individuals that have emerged from different larval hosts (Keena 2002). To eliminate these environmental differences and to assess between population differences, it was necessary to rear at least one generation on artificial diet. Infested branch sections and larvae on artificial diet (Keena 2005) were both transported under permit to the USDA-Forest Service quarantine facility in Ansonia, CT. Voucher specimens of each population were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT.

General colony rearing methods for adult production are given in the study by Keena (2005). We used the wheat germ-based agar diet designated 'AG2' described in the study by Keena (2005) for larval rearing. Newly emerged adults were held in the dark for 4–5 d to allow their exoskeleton to sclerotize before being weighed and fed. Virgin adults of both sexes were held individually in 950-ml glass jars and provided fresh *Acer saccharum* Marshall (sugar maple) twigs (3–7 mm diameter with leaves removed) weekly as a food source. Adults were held at 25 ± 2°C, 60 ± 5% RH and 16:8 (L:D) hr. After the behavioral assay, females were held individually in a 3.8-liter glass jar and weekly provided fresh *A. saccharum* twigs for food and a bolt (3–7 cm diameter and 20 cm long) with both ends waxed as an oviposition substrate. Males were returned to their 950-ml glass jars and fed as previously described until death. Fresh *A. saccharum* twigs and bolts, obtained bi-weekly and monthly, respectively, were stored at 10°C and ≥80% RH until used.

The oviposition bolts were removed weekly from jars containing mated females, and held at the adult holding temperature for 4–5 d before the eggs were removed from under the bark. Eggs were individually placed in labeled wells of a 24-well tissue culture plate that was held in a water box at the adult temperature until hatch. Lifetime fecundity and percentage hatch of eggs produced by each mated female were determined.

Single-Pair 6-hr Behavioral Observation Protocol

To evaluate the reproductive behaviors, five males each from the Chicago, Bayside, and Hohhot City populations were observed individually mating with three separate virgin females. Each male was paired to a new virgin female of similar age from the same geographic population (one exception) when the male was ~2, 4, and 6 wk old. This means that there were 15 pairs from each geographic population and a total of 45 pairs observed. Each pair was placed in a 3.8-liter glass jar with an *A. saccharum* bolt (3–7 cm diameter and 20 cm long) and 2–3 twigs. The temperature was maintained at ~24°C and lighting was both overhead fluorescent and sunlight through a window. The female was placed in the jar first and allowed to settle before the male was added. Pairings started between 7:30 am and 10:40 am (except for one that started at 2:00 pm) and were observed for 6 hr or until natural separation occurred. We were not able to observe pairs until natural separation since males have been reported to remain with females up to 33.5 hr (Morewood et al. 2004). Chewing sites and suspected oviposition sites on each bolt were mapped and once the bolt was removed from the jar they were numbered. The site number was recorded for each egg so that actual oviposition timing within the observation period could be determined. Descriptions of the behaviors and how they were recorded and coded in The Observer XT 11.5 (Noldus Information Technology 2013), are provided in Supp Table 2 (online only). Lag sequential analysis was also performed in Observer XT both within the behavioral groups and across all behaviors regardless of group or sex to develop the sequence of standard behaviors and expected number of occurrences out of one hundred observations.

Statistical Analysis

The fit of the data to various distributions was first evaluated for by using PROC UNIVARIATE (SAS Institute 2015). The Shapiro–Wilk and the Anderson–Darling tests were used to assess normality. All continuous variables were analyzed in PROC GLIMMIX (SAS Institute 2015) using a completely randomized design with male population, female sequence number (1 = first, 2 = second, or 3 = third female mated to the same male), and the interaction of the two as the fixed effects. The only exceptions were the time it took a female to chew an oviposition pit and to deposit an egg because there were too few occurrences to evaluate female sequence number. A normal distribution with an identity link was used for the majority of the variables, but a gamma distribution with a log link was needed for time in copula, total time the male wagged his antennae, the duration of individual male antennal wags, number of copulation attempts, time the female took to chew a pit, time the female took to deposit an egg, and duration of aedeagal bridges. A Beta distribution with a logit link was used for all percentage data. When the number of aedeagal bridges per bout (copulation attempts) was evaluated, the sequential bout number was included as a random effect. We also assessed the effect of bout number on copulation attempts using a gamma distribution with a log link and included population as a random effect. 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). All linear regressions and chi-square tests were conducted using Statistix 10 software (Statistix 2013).

Results

Subject Weights

The weights of females and males used in the observations differed significantly by population (Table 2) but not by female sequence number (females: $F = 0.11$, df = 2, 36, $P = 0.8989$; males: $F = 0.00$, df = 2, 36, $P = 1.0000$) or the interaction between the two (females: $F = 0.29$, df = 4, 36, $P = 0.8837$; males: $F = 0.00$, df = 4, 36, $P = 1.0000$). Females from the Bayside population were significantly smaller than females from the other two populations, while males from the Hohhot City population were significantly larger than males from the other two populations.

Initial Contact and Mounting Behaviors

Reproductive sequences, which were generally typical of lamiines, are summarized in detail in Fig. 1. The majority of males walked around the arena searching for a female while females would walk or settle and feed on the bark of the twigs. When a male antennated a female's antennae or other body parts he would either pursue her if she moved away or make additional contacts with his antennae. On average, males made three contacts before mounting (Table 1) and there were no significant effects of either population (statistics in table) or female sequence number on the number of contacts ($F = 2.3$, df = 2, 36, $P = 0.1153$). Females found and made the first contact with the males 19% of the time, but mounting did not occur without a subsequent male contact.

The time it took the male to find and mount the female once he was placed in the arena did not differ significantly by population or female sequence number (Table 1). Once a male caught a female he would quickly mount generally from behind (without any prior courtship behavior) and 64% of the time attempted to mate immediately. After mounted, the male grasped the female with his pro- and mesothoracic tarsi when attempting to mate or with only his prothoracic tarsi when in amplexus, always keeping his metathoracic tarsi on the substrate (Fig. 2A and C). During the 6-hr observations a prolonged pair-bond was formed, which lasted on average 3.5–4.5 hr and did not vary significantly by population or female sequence number (Table 1). The pair-bond terminated in 40% of the cases when the female successfully forced the male off her back and

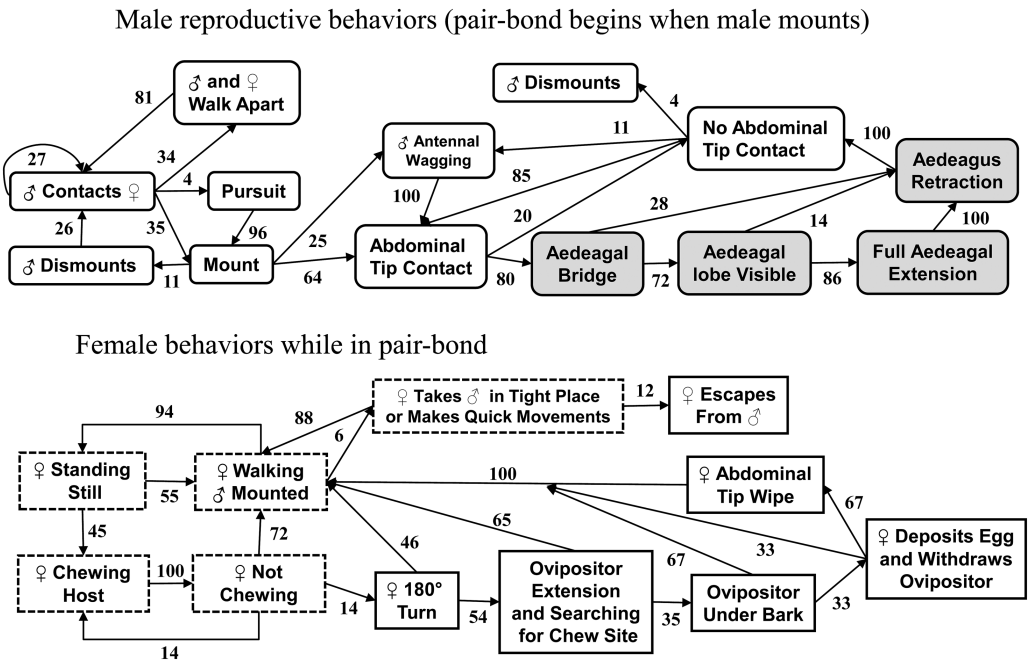

Fig. 1. Male and female reproductive behavioral sequences with the percentage of the time that a particular behavior followed the previous behavior during the 6-hr observations. Female behaviors (bottom) that occurred only when the male was not in copula (shaded boxes at top) appear in text boxes with solid lines. Female behaviors in text boxes with dashed lines occurred any time during the pair-bond.

Table 1. Male mounting behavioral traits in *A. glabripennis* and adult weights (Mean ± SE) by population during the 6-hr observations

Population	Number of contacts before mounting	Time from pairing to mounting (min)	Time male remained mounted (min)	Female weight (g)	Male weight (g)
Bayside	3.4 ± 0.4a	94 ± 17a	206 ± 22a	1.41 ± 0.07b	1.12 ± 0.08b
Chicago	3.4 ± 0.4a	75 ± 17a	280 ± 22a	1.75 ± 0.07a	1.06 ± 0.08b
Hohhot City	2.5 ± 0.4a	52 ± 17a	257 ± 22a	1.75 ± 0.07a	1.41 ± 0.08a
Statistics	F = 0.42, df = 2, 36, P = 0.6604	F = 1.83, df = 2, 36, P = 0.1748	F = 1.04, df = 2, 36, P = 0.3655	F = 6.99, df = 2, 36, P = 0.0027	F = 4.93, df = 2, 36, P = 0.0128

Means within a column followed by the same letter are not significantly different at $P < 0.05$ using Tukey–Kramer post hoc test.

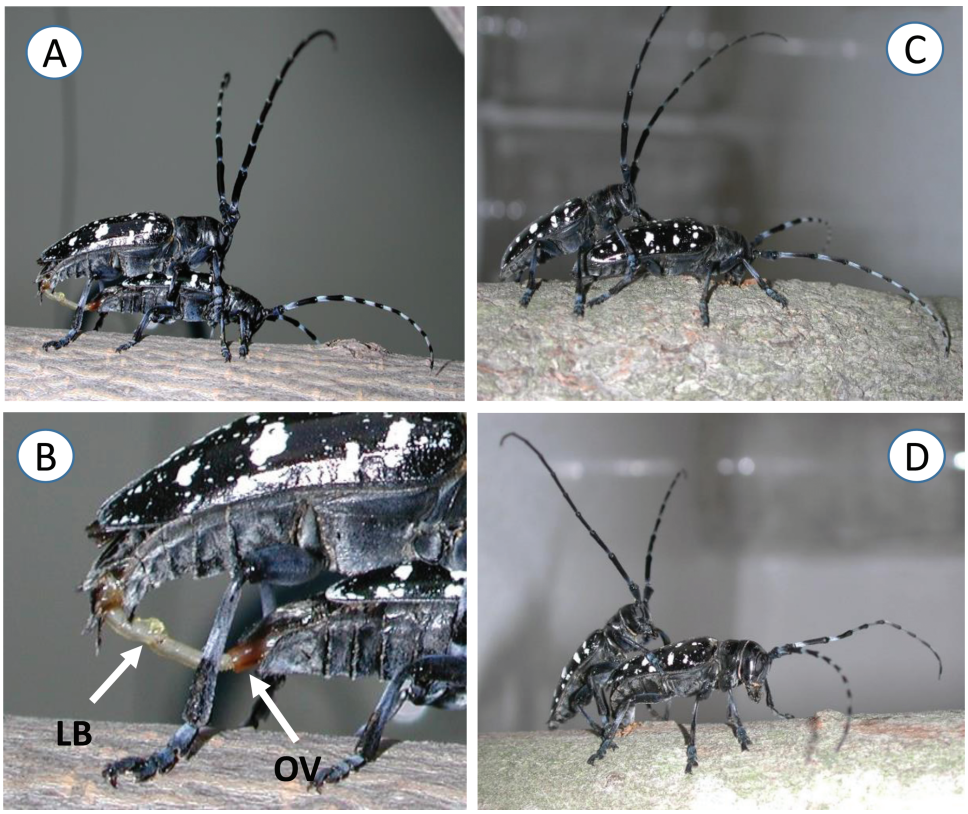

Fig. 2. *Anoplophora glabripennis*: (A) pair in copula showing male position on the female back, (B) close up of pair in copula showing the setose lobe (LB) of the basal segment of the male aedeagus and female ovipositor extended (OV), (C) pair with male in mate guarding position while the female chews an oviposition site, and (D) pair with male in mate guarding position while the female oviposits.

ran away, in 9% of the cases when the male dismounted and walked away, and in 51% of the cases when the observer forced the male to release the female (which quickly walked away) at the end of 6 hr.

Female Receptivity and Male Behavioral Responses

If the female was receptive, the male would attempt to mate with her immediately after mounting. Receptive females generally stopped walking, began chewing on the host and some raised their abdomen. Nonreceptive females would exhibit one or more of the following behaviors on male attempts to mount: ran away, kicked with hind legs, lashed him with her antennae, made quick turns, walked with him mounted through tight places to push him off her back, fell, or flew. Nonreceptive females would also refuse access to their genital chamber by holding the terminal abdominal tergites and sternites tightly together. In these cases, the male might abandon his attempt to mount and mate (11%) or perform a short antennal wagging behavior until the female stopped moving (25%). Females frequently became nonreceptive again after initial copulations and males would again perform the antennal wagging behavior. In 62% of the 29 pairs where females became nonreceptive after copulation, they cleaned their antennae immediately before exhibiting the previously described nonreceptive behaviors. When first mounted, about 50% of the younger females in the first pairing were receptive but a significantly smaller percentage of the older females in the second and third pairings were receptive (Fig. 3). Post copula, a significantly larger percentage of younger females, remained receptive than did older females (Fig. 3).

We did not observe much palpating of the female elytra by the male while in copula or amplexus but the view was often obscured in this setup and the hairs making up the white spots on the elytra were often removed by males if they were kept with females for long periods of time. In 6 of the 45 pairs, the male was observed to quickly jump forward and bite the female's prothorax when she was exhibiting strong nonreceptive behaviors. The male behavior was most frequently observed when the female was nonreceptive, was the antennal wag. Males also often wagged their antennae after

the female completed oviposition and at the end of longer copulatory bouts. The antennal wag lasted on average of 24–31 s and was repeated four to six times during the time pairs were bonded (Table 2). The number of times the male wagged his antennae and the duration of individual antennal wag periods did not differ significantly by population (see Table 2 for statistics) or female sequence number (number wags: $F = 0.7$, df = 2, 36, $P = 0.5044$; length of individual wags: $F = 0.32$, df = 2, 36, $P = 0.7278$). The total time the males wagged antennae differed significantly by population with males from the Hohhot City population wagging antennae longer than those from the Bayside population (Table 2).

Copulation and Associated Behaviors

The male copulatory behaviors and female behaviors that occurred simultaneously or between copulations are summarized in Fig. 1 and shown in Fig. 2A and B. One-quarter of all attempts to copulate consisted of the creation of an aedeagal bridge that generally terminated in ≤1 min (Fig. 4A). These short copulatory attempts occurred most frequently just after mounting and at the beginning of bouts. During these times, the female was less receptive and the male appeared to be either unable to enter the female genital opening or to engage and inflate the endophalus to maintain the connection. Insemination did not occur unless the aedeagal bridge lasted ≥1 min (range 1–17 min) and the aedeagus was fully extended (Fig. 4A).

Males mated with females in a series of copulatory bouts with refractory periods between them. Neither female sequence number nor its interaction with population had a significant effect on any of the parameters associated with copulation. The total number of copulatory attempts varied significantly with bout number ($F = 4.70$, df = 4, 141, $P = 0.0014$), the first bout having more than the third or subsequent bouts (Fig. 4B). However, the number of copulations (with the aedeagus fully extended) per bout did not vary significantly by bout number. The number of copulatory bouts during the pair bond averaged 3–4 regardless of population. The average number of aedeagal bridges per bout, however, did vary significantly by population with males from the Hohhot City population having more than the other two populations (Table 3). Refractory periods between bouts did not vary significantly with population, but males from the Bayside population tended to take longer between bouts than males from the other populations (Table 3). Males from Bayside completed significantly fewer copulations (also fewer total attempts) than males from the Hohhot City population. Males from the Bayside population also had a significantly shorter total time in copula than males from the other two populations, although the average time spent in individual copulations did not vary significantly by population (Table 3).

During the copulation, the female ovipositor would be extended 29% of the time when the male aedeagus was fully extended. After longer copulations, the females kept their ovipositor extended 39% of the time and tapped it on the substrate leaving a clear fluid. This fluid contained no particulate matter when viewed under a microscope. The chemical composition of the fluid and purpose of this action is unknown.

Oviposition and Associated Behaviors

Females chewed oviposition pits during or between copulation events when the male was in a half-mount position as shown in Fig. 2C. Female sequence number and its interaction with population did not have a significant effect on any of the parameters associated with oviposition so the data in the tables will only be presented by population. Females from the Chicago population chewed significantly more sites on the host than did females from the Bayside population and those from the Hohhot City population chewed an intermediate number of sites (Table 4). However, the percentage of chewed sites where the female attempted to deposit an egg (~one-third) and oviposition attempts where an egg was actually deposited did not vary significantly by population (Table 4). The time it took females to chew the oviposition sites did not vary significantly between populations (Table 4).

When a female completed chewing the oviposition site, she would make a 180° turn with the male in tow and then search for the site with her ovipositor. The oviposition site was a slit on thin bark and a conical pit in thick bark. Once she found the center of the oviposition site, she would use her last abdominal tergites and sternites to pry an opening by rocking back and forth and then insert her ovipositor (Fig. 2D). Under the bark she created an oval opening above the chew site where she inserted the egg. When the bark was removed to retrieve eggs, the oval area surrounding each egg was discolored and the tissue was apparently dead likely due to wound response or a female secretion. Thinner bark often split when the female deposited an egg. Females inserted a single egg and, on rare occasions two, into the site created under the bark and the time required for oviposition was 8–11 min (Table 4). Two-thirds of the time after an egg was deposited (rarely otherwise), the female would seal the opening in the bark with a gelatinous material and then she would wipe the tip of her abdomen back and forth over the site rubbing frass into it. Sites where eggs had been deposited would eventually become obscured by fungal growth when the bolt was moist enough to ooze sap. Once the female oviposited she walked palpating the bark until she found an acceptable location and again began chewing the host. Males occasionally tried to copulate during oviposition but were generally not successful unless the female abandoned a site.

Overall, 40 eggs (0–5 per female) were deposited by the females while being observed (all female sequence numbers combined), 28 of which hatched. Three of the first or second females mated to the male and six of the third females mated to the male oviposited while in pair-bond. The youngest female that oviposited was 21 d post eclosion. One older female from the Hohhot City population deposited an infertile egg 43 min before the male mounted her. The average time from mounting to the first oviposition was 117 ± 17 min (range 53–209 min). On average, the female oviposited after chewing

Table 2. Male *A. glabripennis* antennal wagging behavior (Mean ± SE) by population during the 6-hr observations

Population	Number of times male wagged antennae	Duration of male antennal wags (s)	Total time male wagged his antennae (min)
Bayside	3.9 ± 0.8a	24 ± 2.9a	1.7 ± 0.3a
Chicago	5.8 ± 0.8a	27 ± 2.6a	2.6 ± 0.4ab
Hohhot City	6.0 ± 0.8a	31 ± 3.0a	3.0 ± 0.5b
Statistics	$F = 1.94$, df = 2, 36, $P = 0.159$	$F = 1.1$, df = 2, 227, $P = 0.3353$	$F = 3.31$, df = 2, 36, $P = 0.0477$

Means within a column followed by the same letter are not significantly different from each at $P < 0.05$ using Tukey–Kramer post hoc test.

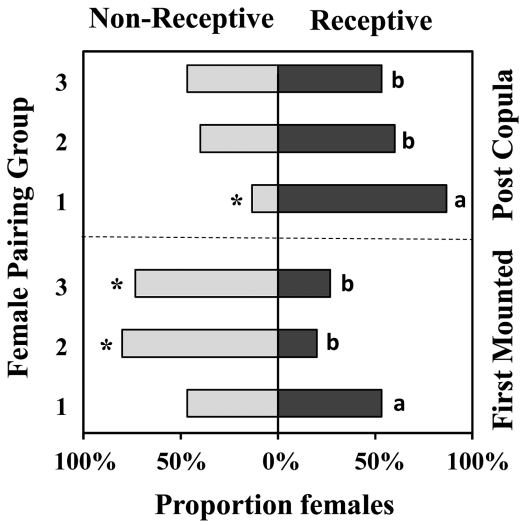

Fig. 3. Comparison of the proportion of *A. glabripennis* females that were receptive when first mounted and after initial copulations by female sequence number group. Bars followed by different letters within a time frame were significantly different and bars preceded by * indicate that there were significant responses based on chi-square tests at α =0.05.

4.6 ± 0.6 (range 2–9) sites, and first oviposition occurred most often during the first or second copulatory bout (one in the fourth). The shortest time from the first copulation to first fertile egg deposited was 91, 42, and 57 min for females from the Bayside, Chicago, and Hohhot City populations, respectively.

Female lifetime fertility did not vary significantly by population, female sequence number or the interaction between the two (Table 4). There was, however, a significant inverse relationship between lifetime female fecundity and female age at mating (Fig. 5). The older the female at the time of first mating the fewer eggs she deposited, hence the third females mated to each male tended to deposit fewer eggs than the first and second females.

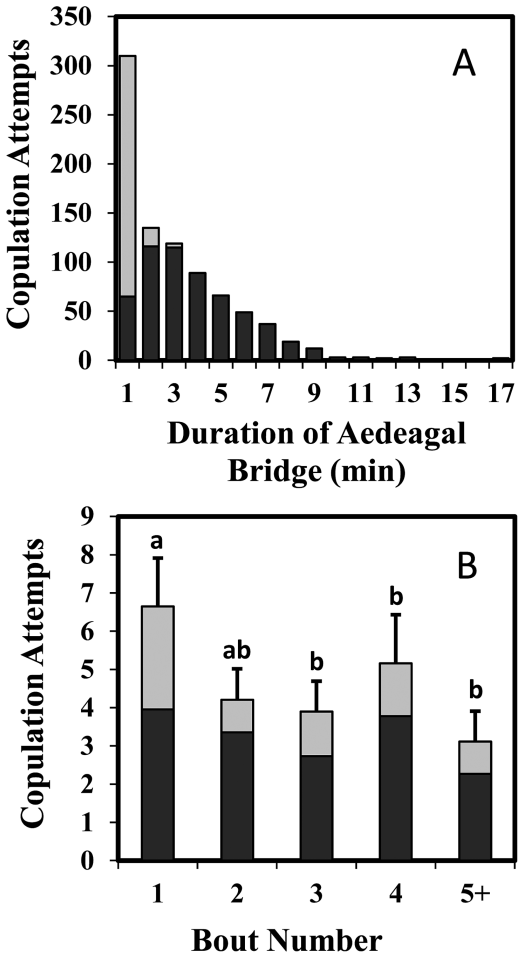

Fig. 4. *Anoplophora glabripennis*: (A) aedeagal bridge duration (category 1 is all bridges ≤1 min) frequencies combined for all three populations, (B) average (± SE) number of copulation attempts per copulatory bout for all pairs combined. In each graph the light gray portion of each bar represents the attempts without full aedeagal extension and the black represents those with full extension.

Discussion

Anoplophora glabripennis reproductive behaviors are similar to those described for other diurnally active cerambycids in the subfamily Lamiinae. The isolated pairs that we observed formed prolonged pair-bonds and males had sufficient time in copula to transfer enough sperm for the female to achieve the same average lifetime fertility (~60%) as a female held with a male for life (Keena 2006). Females oviposited between copulatory bouts and were able to deposit fertilized eggs quickly. There were some significant differences between the populations in parameters associated with both male antennal wagging and copulatory behaviors, and in the number of chew sites the female made during the pair-bond. Female age at mating was also negatively correlated with lifetime fecundity and female receptivity changed with age and mating status.

Female *A. glabripennis* played an active role in mate finding, mate acceptance, and determining the duration of the pair-bond. Some virgin female *A. glabripennis* approached and made first contact with the male as has been reported for two other lamiines, *Monochamus alternatus* Hope (Fauziah et al. 1987) and *A. malasiaca* (Fukaya et al. 2005). This behavior is in response to the male produced pheromone, but mounting and copulation did not proceed until the male made contact with the sex-specific contact pheromone on the female's body. However, females often responded to male contact with avoidance or rejection behaviors upon mounting as is common in many species of cerambycids (Hanks and Wang 2017). The higher percentage of precopulatory rejection in older *A. glabripennis* females than in younger females is opposite of what occurs in *Psacothea hilaris* (F.) (Coleoptera, Cerambycidae) (Yokoi 1989), where younger females are less receptive possibly due to not yet having mature eggs (Thornhill and Aklcock 1983). Postcopulatory rejection was observed in a higher percentage of older *A. glabripennis* females than in younger females, possibly because of differing fitness advantages. Younger females would potentially increase fitness and maximize their fertility through additional copulations to maximize sperm transfer, while older females may maximize fitness through uninterrupted oviposition given that their lifetime fecundity is dependent on how fast they can deposit eggs before they die. Postcopulatory rejection is common in the Lamiinae (Hughes 1981; Wang et al. 1990, 1996b; Kobayashi et al. 2003) and along with interference from other individuals (especially males) is a major factor in determining the duration of the pair-bond. When the female remained nonreceptive after antennal wagging the male would either leave or the female would perform behaviors that forced the male off her back and ultimately ended the pair-bond.

Table 3. Description of male *A. glabripennis* mating boughts and copulations (Mean ± SE (n)) by population during the 6-hr observations

Population	Number of copulatory bouts	Number of aedeagal bridges per bout	Total number of aedeagal bridges*	Average duration of copulations† (min)	Total number of copulations†	Total time in copula (min)	Refractory period between bouts (min)
Bayside	2.7 ± 0.4a	4.6 ± 0.6a	11.4 + 1.3a	3.2 ± 0.2a	7.7 ± 1.3a	27 ± 3a	70.9 ± 6.0a
Chicago	3.9 ± 0.4a	3.7 ± 0.4a	15.9 + 1.3ab	3.1 ± 0.2a	11.9 ± 1.3b	39 ± 4b	54.0 ± 5.1a
Hohhot City	3.3 ± 0.4a	7.0 ± 0.8b	23.7 + 1.3b	3.2 ± 0.2a	13.6 ± 1.3b	47 ± 5b	57.6 ± 5.5a
Statistics	$F = 2.57$, df = 2, 36, $P = 0.0906$	$F = 7.7$, df = 2, 141, $P = 0.0007$	$F = 9.1$, df = 2, 36, $P = 0.0006$	$F = 0.28$, df = 2, 498, $P = 0.7535$	$F = 5.87$, df = 2, 36, $P = 0.0062$	$F = 7.86$, df = 2, 36, $P = 0.0015$	$F = 2.44$, df = 2, 100, $P = 0.0922$

Means within a column followed by the same letter are not significantly different from each at $P < 0.05$ using Tukey–Kramer post hoc test.
*This counts all occurrences of the formation of an aedeagal bridge regardless of duration.
†Only aedeagal bridges with full extension were considered copulations and counted here.

Table 4. Female *A. glabripennis* oviposition behaviors and fertility (Mean ± SE) by population during the 6-hr observations

Population	Number of sites on the host the female chewed during the pairing	Percentage of host chewing that resulted in an oviposition attempt	Percentage of oviposition attempts that eggs were deposited	Time female took to chew an oviposition site (min)	Time female took to deposit an egg (min)*	Lifetime percentage fertility
Bayside	6.2 ± 1.3b	36.4 ± 8.5a	27.6 ± 12.6a	8.3 ± 1.4a	9.5 ± 2.3a	58.9 ± 5.4a
Chicago	10.9 ± 1.3a	30.0 ± 7.9a	42.8 ± 12.9a	8.0 ± 1.1a	8.4 ± 1.7a	50.1 ± 5.4a
Hohhot City	8.1 ± 1.3ab	23.4 ± 7.9a	57.9 ± 14.1a	5.0 ± 0.8a	11.3 ± 2.5a	57.1 ± 5.4a
Statistics	$F = 3.12$, df = 2, 36, $P = 0.0565$	$F = 0.76$, df = 2, 36, $P = 0.4751$	$F = 1.73$, df = 2, 16, $P = 0.2095$	$F = 3.28$, df = 2, 37, $P = 0.0488$	$F = 0.48$, df = 2, 37, $P = 0.6243$	$F = 0.8$, df = 2, 36, $P = 0.4556$

Means within a column followed by the same letter are not significantly different from each at $P < 0.05$ using Tukey–Kramer post hoc test.
*This was calculated as the time from when the female made a 180° turn until she withdrew her ovipositor from under the bark.

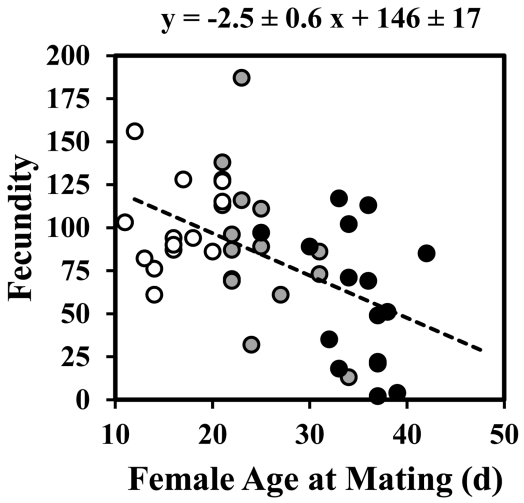

Fig. 5. Relationship between *A. glabripennis* age at mating and lifetime female fecundity after one extended pair bond with multiple copulations (dashed line and equation above graph). Data points with no fill from first pairing, with gray fill from second pairing, and with black fill from third pairing with the males used in the 6-hr observations.

Male *A. glabripennis* exhibited behaviors characteristic of female defense polygyny, which occurs when the host resource is too big to monopolize and the females are the limiting resource (Thornhill and Aklcock 1983). Males generally mounted females after initial contacts, remained with the female postinsemination, and stayed with the female while she oviposited. Pair-bond durations similar to those reported here for *A. glabripennis* have been reported for other lamiines: *Glenea cantor* (F.) (2.5–3.5 hr), *A. chinensis* (mean 5.9 hr), and *M. alternatus* (3.5 hr) (Wang et al. 1996a, Togashi 1998, Lu et al. 2013). Copulatory bouts with oviposition occurring in-between has also been seen in *A. chinensis* and *Monochamus scutellatus* (Say) (Hughes 1981, Wang et al. 1996a). Several benefits have been suggested for these types of behaviors, such as reducing female harassment by other potential mates, preventing a rival's sperm from being used, and achieving additional copulations to maximize sperm transfer (Alcock 1994, Wang et al. 1996a). There are also costs to remaining with a mate, such as lost time in acquiring additional mates, thus reducing the spread of their genes in the population. However, the short time from copulation to oviposition as seen in *A. glabripennis* is known to increase mate guarding in other species so female receptivity will ultimately dictate the duration of the pair-bond (Alcock 1994).

Removal of competitor sperm through short aedeagal bridge formation was documented for another lamiine *P. hilaris* (Yokoi 1990). *A. glabripennis* does form several <1 min aedeagal bridges, especially during the first bout, which is characteristic of the sperm removal behavior in *P. hilaris*. Although we do not have any evidence to rule out sperm removal, these short bridge formations in *A. glabripennis* occurred more often when the female was not receptive and the male did not seem to be able to gain access to the female's genitalia. Females have extensive musculature surrounding their reproductive tract that are needed for prying up the bark to deposit eggs and this musculature appears to also allow the female to restrict access to her gonophore as is suspected to occur in many Coleoptera (Eberhard 1991). Male antennal wagging which increases female receptivity (they stop moving) may also provide the female with cues about male fitness, which she uses in determining whether to allow him to copulate or not. More vigorous males may wag at a faster speed or for longer, and larger males may brush her entire antennal length during the wag while smaller ones may not. Further research would be needed to assess whether characteristics of the male antennal wag are correlated with male fitness and female mate choice.

It is possible that behavioral differences among populations may be associated with beetle size. Larger males from the Hohhot City population wagged longer and formed more aedeagal bridges per bout than the smaller males from the Bayside population that also had fewer copulations and the total time in copula was shorter. However, size was correlated with population in this study and there is insufficient male variation within any one population to evaluate size separately. There did not appear to be any difference in female fertility associated with the reduced copulatory time for pairs from the Bayside population so the fitness implications are uncertain. Likewise, the fitness ramifications are uncertain for the larger females from the Chicago population that chewed more oviposition sites, started to oviposit sooner, and deposited eggs in a higher percentage of pits than the smaller females from the Bayside population. However, it has been shown that the females from the Chicago population deposit more eggs during the peak oviposition period than New York females which may be an adaption to accommodate the shorter growing season in Chicago compared with New York City (Keena 2002).

As eradication efforts cause the populations of *A. glabripennis* to decline in invaded areas, the beetle's reproductive behaviors will tend to increase the rate of population density decline. Males and females will likely find each other if they are on the same host tree since both sexes produce pheromone that aid in mate finding; males produce a pheromone to attract virgin females (Zhang et al. 2002) and as they walk, females deposit a sex trail pheromone that males follow (Hoover et al. 2014). As observed in this study, once they find each other pairs will stay together long enough to ensure the female can fertilize ~60% of the eggs she deposits. However, the sexes may not emerge from the same tree since many trees in eradication zones have only one exit hole (Trotter and Hull-Sanders 2015) or at the same time since males tend to emerge first (MAK, unpublished data) and it could take a while for them to find each other. As shown here, delayed mating will reduce the number of offspring which will tend to reduce population densities. In addition, some virgin females may never find a mate as demonstrated by late summer virgin females being caught in traps (Nehme et al. 2014), which also reduces population density. Lower densities, however, may reduce mate guarding and assortative mating as male search time for a mate increases ultimately resulting in a higher percentage of male mountings that produce viable offspring as documented for other beetles (Mclain and Boromisa 1987).

There are still several unanswered questions that would further aid in understanding *A. glabripennis* reproductive behaviors and their role in population dynamics. Can females achieve a higher percentage fertility mating with multiple males or with matings spread out over her lifetime? How many females can a single male successfully inseminate? How does the presence of other conspecifics affect a pair-bonded couple? Will an aggressive encounter with a conspecific result in beetles spreading themselves out more on the host or dispersing to other hosts? Further work on the reproductive behaviors of *A. glabripennis* would provide more information that is critical to managing this insect and achieving eradication in invaded areas.

Supplementary Data

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

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