

## Field observations of visual attraction of three European oak buprestid beetles toward conspecific and heterospecific models

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### Abstract

*Agrilus biguttatus* Fabricius, *Agrilus sulcicollis* Lacordaire, and *Agrilus angustulus* Illiger (Coleoptera: Buprestidae) are three beetle species associated with oak trees [*Quercus* spp. (Fagaceae)] in Europe. In Hungary, all three species were observed in the foliage near freshly cut oak log piles. *Agrilus biguttatus* was active later in the afternoon, whereas the other species were observed earlier in the day. Dead female models of these three native *Agrilus* species, as well as the native species *Agrilus cyanescens* Ratzeburg and the non-native *Agrilus planipennis* Fairmaire, were pinned onto adjacent leaves in direct sunlight to observe the visual mating approaches of the local male populations. *Agrilus biguttatus* and *A. sulcicollis* males flew toward and landed directly on the models from a distance of 1 m. *Agrilus angustulus* flew toward the models from a similar distance, but rather than landing directly on a model would alight on the leaf, 1–2 cm away, before walking closer to the model while antennating it. For all three species, there was substantial cross-attraction to models of other species. Both *A. biguttatus* and *A. sulcicollis* males chose *A. angustulus* models less often than their respective conspecific models. Likewise, *A. angustulus* males approached *A. sulcicollis* models less often than their normal conspecific models. *Agrilus biguttatus* males attempted to copulate with both *A. biguttatus* and *A. planipennis* models, afterward remaining with them for several minutes. *Agrilus biguttatus* males spent more time on *A. planipennis* models than on conspecific models. Thus, there is substantial cross-species attraction in visually mediated mating approaches and copulation behavior. These findings suggest a common behavioral template for visual mate-finding among buprestids and a large degree of close-range mating compatibility between *A. biguttatus* and *A. planipennis*.

### Introduction

The threat of buprestid beetles as pests has been underscored by the recent invasive spread in North America of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), which has caused substantial mortality of ash trees and may potentially impact large forest areas (MacFarlane & Meyer, 2005; Poland & McCullough, 2006). Some buprestid species, such as *Agrilus*

*biguttatus* Fabricius and *Agrilus viridis* L., are considered to be severe forest pests in their native range (Moraal & Hilszczanski, 2000; Molnár et al., 2010). Others recently have spread outside of their normal host ranges, both within and between continents, to become more severe pests (Moraal & Hilszczanski, 2000; Coleman & Seybold, 2008; Haack et al., 2009; Jendek & Grebennikov, 2009). One European oak-feeding buprestid species, *Agrilus sulcicollis* Lacordaire, has recently been identified in Canada and the USA (Haack et al., 2009; Jendek & Grebennikov, 2009), prompting concern that this species and the more aggressive *A. biguttatus* might threaten the oak forests of North America.

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Despite this increasing problem of invasive buprestid species to forest ecosystems, very little is now understood about their host-finding and mating behaviors, limiting the development of potential detection and management tools. Among these species, *A. planipennis* has been most intensely researched. Traps for detecting *A. planipennis* have been introduced following its discovery in North America (Francese et al., 2005), accompanied by continuing efforts aimed at gaining insights into the behavior of the beetles for improved trapping (de Groot et al., 2008; Lelito et al., 2007; Crook et al., 2009; Francese et al., 2010; Grant et al., 2010).

Studies of *A. planipennis* have recently described attraction to host volatiles (Rodriguez-Saona et al., 2006; Crook et al., 2008; de Groot et al., 2008; Grant et al., 2010), strong visual mate-finding behavior in the field (Lelito et al., 2007), and possible pheromones that operate at close range (Bartelt et al., 2007; Pureswaran & Poland, 2009). The visual mate-finding behavior described for *A. planipennis* involves a swift direct flight from approximately 1 m above dead pinned conspecific specimens (Lelito et al., 2008). Such flights occur toward models of either sex. Neither the gender of the model nor washing of surface chemicals from the models affects the frequency of such approaches. Furthermore, the rapid direct trajectory of these flights, with an orientation independent of wind direction, precludes the possibility that any odors emanating from the female are tracked in the execution of this behavior. After making such a flight, the wild males will try to copulate with models of either gender. However, the duration of such copulatory contact is affected by gender and the presence of cuticular hydrocarbon contact sex pheromone components (Lelito et al., 2009; Silk et al., 2009). This rapidly unfolding behavioral sequence of direct flight onto the models followed by copulation attempts was described as ‘paratrooper copulation’ (Lelito et al., 2007).

Similar visually mediated direct approaches toward dead models have also been observed in the tree-dwelling buprestids *Agrilus cyanescens* Ratzeburg and *Agrilus subcinctus* Gory (Lelito et al., 2011). For *A. cyanescens*, it was further discovered that such paratrooper copulations also occur if heterospecific models were used. The alternate models tested ranged from *A. planipennis* to isolated elytra extracted from the tiger beetle, *Cicindela sexguttata* Fabricius. The frequency of responses to the tiger beetle elytra was not different from that to conspecific models (Lelito et al., 2011).

The prevalence of such behavior across buprestid species is not known, nor has the degree of cross-species attraction been explored. Here, we investigate aspects of the mating behavior of three European buprestid species,

*A. biguttatus*, *A. sulcicollis*, and *Agrilus angustulus* Illiger, that are commonly found feeding sympatrically on oak [*Quercus* spp. (Fagaceae)]. All three species can appear green, but have some differences in their iridescence patterns that are observable to the human eye (Figure 1). The coloring of *A. biguttatus* tends to show hints of black and yellow, *A. sulcicollis* often has a bluish cast, and *A. angustulus* can appear somewhat red or brown. *Agrilus biguttatus* can be further distinguished by white spots on the elytra and abdomen. The life cycles of all three oak buprestid species are similar to those described for other tree-feeding buprestids such as *A. planipennis*. Female adults lay their eggs in bark crevices, with larvae spending 1–2 years feeding on the cambium and sapwood before pupating in the spring and emerging as adults during May through July (Schimitschek, 1944; Schaefer, 1949; Wachtendorf, 1955; Bílý, 1982, 2002). There is evidence that warm, dry summer weather helps to promote the growth and reproduction of *A. biguttatus* (Schlag, 1992). Mass occurrences of this species are often recorded 1–2 years after outbreaks of defoliating insects such as *Lymantria dispar* L., causing further decline of trees weakened by the defoliation (Csóka & Kovács, 1999; Csóka & Hirka, 2006; McManus & Csóka, 2007). In contrast, *A. sulcicollis* and *A. angustulus* are not as strongly associated with oak decline (Schopf, 1992).

The extent and nature of competitive interactions between these species have not been fully explored, but differences in host utilization have been noted. The smaller species *A. sulcicollis* and *A. angustulus* (Figure 1) attack smaller branches and the upper parts of stems and smaller trees (Kolk & Starzyk, 1996; König, 1996; Csóka & Kovács, 1999; Muskovits & Hegyessy, 2002). The largest of the three species, *A. biguttatus* (Figure 1), attacks larger diameter stems and branches and has been linked to oak decline and mortality (Moraal & Hilszczanski, 2000; Vansteenkiste et al., 2005). Thus, the differences in host utilization patterns are analogous to those observed in scolytid bark beetle species (Reid, 1955), whose conflicting competitive and cooperative interactions are mediated by a complex array of mechanisms involving host volatiles, pheromones, and flight phenology (Birch et al., 1980; Paine et al., 1981; Ayres et al., 2001).

A greater understanding of the behavior of *A. biguttatus*, *A. sulcicollis*, and *A. angustulus* is necessary to develop tools for their detection and control. Here, we examine whether these species exhibit visual mate-finding behaviors similar to those observed in *A. planipennis*, *A. cyanescens*, and *A. subcinctus*. Such information is needed for a preliminary assessment of the potential for developing visual trapping approaches for these insects. From this perspective, it is also of interest whether the foraging males of each oak buprestid species exhibit cross-attraction toward

the other species. Thus, we also report the degree of attraction among the various local oak-feeding species to each other, as well as to additional species that are normally found feeding on other hosts. As buprestid populations expand their geographic distributions, knowledge of the compatibility of the visual mating systems may help determine whether one visually-based trapping design might be possible that will be effective for multiple species, or whether different trapping approaches might be needed.

### Materials and methods

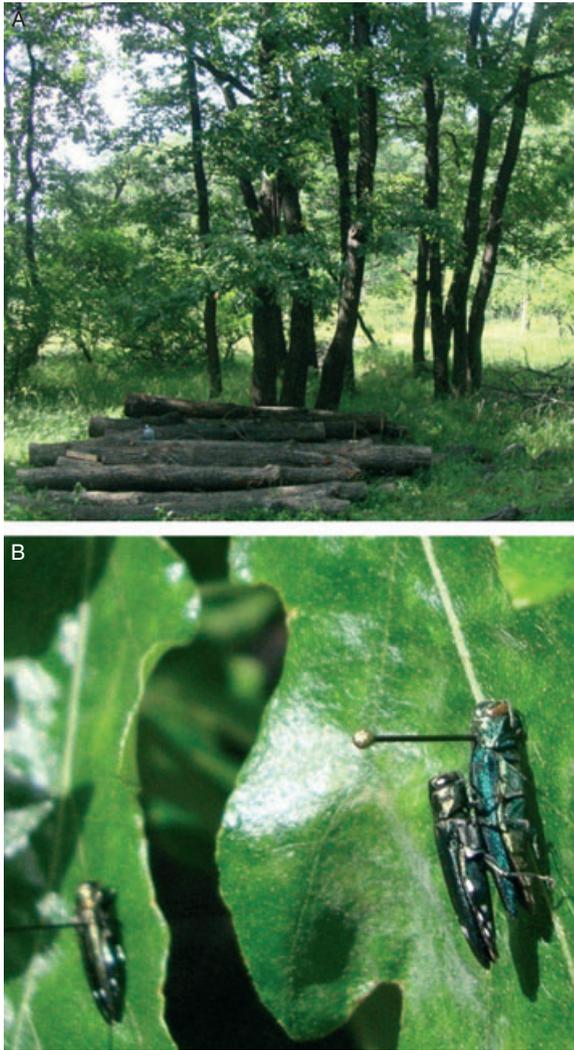
A field site was located near Mátrafüred, Hungary (47°50'17"N, 19°59'50"E), in a mixed oak forest where logging had been active in the past year. Throughout the forest, piles of cut logs had been stacked, both in clearings and near standing trees. We were able to find buprestid beetles of all three species present and occasionally mating on the leaves of south-facing tree branches above log piles. Before beginning an observational study, beetles were collected by sweep-netting the foliage and log piles from such locations for 2 days. Species and sex identifications were performed according to Muskovits & Hegyessy (2002). One particular sessile oak, *Quercus petraea* (Matt.) Liebl., with a substantial amount of foliage 2–3 m from the ground and a 1-m-tall log pile under it, was used for the observational study (Figure 2A). The south-facing branches of this tree were in a clearing that received direct sunlight between 11:30 and 15:00 hours, when beetles could be observed by standing on the log pile below the lowest branches. Formal observations were made over the course of 4 days. The first three of these days were hot and humid with high temperatures at or close to 30 °C. The 4th day (2 days after the last of the warmer days) had a mixture of rain and sun with a high temperature of 25 °C.

To assess the mating behavior of these three species, pinned dead buprestid models were placed on the leaves. Specimens were pinned through the thorax. The use of pins allowed easy application to leaves and the ability to rapidly move samples between observational replications, experimental features that were necessary despite the potential visual and physical distraction caused by the pins. Females of five species were used (Figure 1). These species included the three native oak-feeding species that were being observed, *A. angustulus*, *A. sulcicollis*, and *A. biguttatus*. All of these native models that had been collected in the previous 2 days were frozen overnight at 20 °C before being pinned. Models from two other species that had been collected 4 years earlier in Michigan (USA) were also used. One was the Asian-originating emerald ash borer, *A. planipennis*, which was larger than all of the European oak-feeding species models. Also included was the European-originating species *A. cyanescens*, which is similar in size to *A. sulcicollis*, and for which honeysuckle [*Lonicera* spp. (Caprifoliaceae)] is the primary host (Muskovits & Hegyessy, 2002).

Preliminary observations were made of the behavior of the native species toward models placed on the sunlit surfaces of leaves. *Agrilus biguttatus* could easily be distinguished from the other species based upon their distinct coloration patterns. *Agrilus sulcicollis* and *A. angustulus* were distinguished primarily by observation of size and coloration differences (Figure 1), with light reflectance being greater and the coloration tending more toward blue for *A. sulcicollis*. *Agrilus biguttatus* and *A. sulcicollis* were observed performing the same distinctive 'paratrooper copulations' previously described for *A. planipennis*, whereby there was a direct aerial approach toward models from 1 m away resulting in mounting the female (Lelito et al., 2008). *Agrilus angustulus* performed a similar



**Figure 1** The five buprestid species used as models for attracting European oak buprestids *Agrilus planipennis* (8–15 mm), *Agrilus biguttatus* (10–14 mm), *Agrilus sulcicollis* (6–8.5 mm), *Agrilus cyanescens* (4.5–7.5 mm), and *Agrilus angustulus* (4–6.5 mm). In parentheses the ranges of lengths according to Muskovits & Hegyessy (2002).



**Figure 2** (A) The sessile oak tree (*Quercus petraea*) used for field observations. Pinned specimens were placed on neighboring leaves while standing upon the log piles visible below. Mating approaches were observed while standing on these log piles from a distance of approximately 1.5 m. (B) On this tree, an *Agrilus biguttatus* male is guarding a pinned *Agrilus planipennis* female after a paratrooper approach and an attempt to copulate. Another pinned *A. biguttatus* female model is visible in the background.

approach but landed 1–2 cm from the model, which is behavior similar to that reported for *A. subcinctus* (Lelito et al., 2011). The mate-approaching flights of all three species were observed to occur even when the wind was clearly blowing perpendicular to the flight track. The *A. angustulus* that missed landing on the models would immediately walk toward the model while moving its antennae. Then, these *A. angustulus* males would quickly walk or fly away from the model, but often linger on the same leaf or neighboring leaves. This allowed for the collection and verifica-

tion of species identity in our preliminary observations. For formal observations, no such collections were made for fear of interfering with the observational experiment.

To assess and compare visual attraction to the five models, they were placed on different neighboring leaves, as close to 10 cm apart as allowed by leaf spacing and observed for 10-min periods. Leaf surfaces were chosen that were directly perpendicular to and illuminated by sunlight. Between observation periods, the specimens were re-arranged on a different cluster of leaves at least a half a meter away from the previous cluster. Because of a limited number of specimens, models were used for up to one full day. They were changed sooner only if damaged. The same single observer made all assessments of behavior using one set of models at a time. Within the 4 days of observation, there were approximately 10 such observational intervals on the 1st day and 20 per day on the next 3 days. When successful visual approaches were observed, the following behaviors similar to those reported for other *Agrilus* toward pinned models (Lelito et al., 2007, 2011) were noted: (1) descending onto and landing on a leaf that had a model pinned to it (indirect approach), (2) descending onto and landing directly on a pinned model (direct approach), (3) copulation after landing on a model, which was often directly associated with wing-fanning, and (4) remaining on top of the model after copulation (Figure 2B). The times at which males landed on a particular model and the amount of time spent on the model were also recorded. When males landed on the models for at least 5 s, copulation attempts were always observed. Otherwise, males stayed on the models for <2 s. All observations were recorded at 1-s intervals such that 1 s was the minimum time.

#### Statistical analysis

The temporal patterns of mating flight patterns were assessed by examining the correlations between the numbers of males of each of the three species observed approaching models and time of day the observations were made. A principle component (PC) analysis was performed using Proc PRINCOMP in SAS (Cary, NC, USA) v. 9.2 to elucidate the patterns of *A. biguttatus*, *A. sulcicollis*, and *A. angustulus* mate searching with respect to time.

For comparing the choices made by the field populations of *A. biguttatus*, *A. sulcicollis*, and *A. angustulus*, a log linear model was fit to the data using the Proc CATMOD feature of SAS v. 9.2. This model allowed comparison of the proportion of males landing on each model (or on leaves near particular models in the case of *A. angustulus*), while also assessing the significance of additional experimental factors. Among those factors tested that might impact the choice of model were the observation

period within which the choice was made, the previous choice observed, and the within-period time of the choice. Observations of flights onto a second model when a male was on top of another model were not included in this analysis, because in such a case, the second male would be presented with a biased choice. Contrasts were evaluated comparing the proportion of times the male chose the conspecific model vs. the other four species.

For *A. biguttatus*, which was the only species that copulated with and remained on top of female models, we compared the proportion of copulation events per paratrooper approach among the five models, using Fisher's exact test. As we did for the model of choice preference, we used the conspecific observations as the reference point for our comparisons.

Finally, the average durations of copulation behavior by *A. biguttatus* toward the different types of models were compared by ANOVA using Proc GLM in SAS v. 9.2. To satisfy normality and equality of variance assumptions, the data were log-transformed before analysis.

## Results

### Temporal patterns of activity

Male and female *A. angustulus* were observed maturation-feeding on leaves at all times, often before and after our 11:30–15:00 hours mating-related experimental observation period. *Agrilus sulcicollis* and *A. biguttatus* were also observed on the foliage, but only sporadically and seldom without the pinned models attracting males. *Agrilus sulcicollis* were also readily observed on the log piles, as were *A. biguttatus*, but with less frequency. Beetles collected for use as models included four female and zero male *A. biguttatus*, 14 female and three male *A. sulcicollis*, and more than 25 of each sex of *A. angustulus*. Collection and identification of *A. angustulus* were terminated once it became clear that enough models had been obtained. All three species were observed approaching the pinned models on the first three hotter days, but only *A. angustulus* and *A. sulcicollis* were observed on the last cooler day with intermittent rain and clouds. Although only these three species were positively identified, it should be noted that we performed numerous in situ observations that could not be verified further in the laboratory. For example, *A. angustulus* is of similar size to another European buprestid species, *A. obscuricollis*, which is somewhat darker.

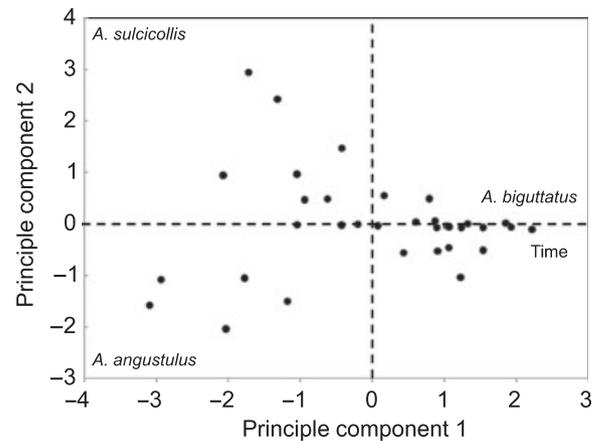
There were 34 formal observation periods over the 4 days in which at least one wild male was observed approaching the model. Per day, this included six, 14, 12, and two respective periods. When no flight approaches were observed in a period, prolonged bouts of cloudiness

were noted, in which direct sunlight did not reach the models. There were 26 *A. angustulus* approaches in 12 of these periods (mean  $\pm$  SE =  $2.17 \pm 0.42$  observations per period; range: 1–5). For *A. sulcicollis*, there were 28 approaches in 12 periods ( $2.33 \pm 0.48$  observations per period; range: 1–6). Finally, there were 123 *A. biguttatus* approaches in 26 of the periods ( $4.73 \pm 0.66$  observations per period; range: 1–13).

Principle component analysis provides a means of describing the temporal patterns of the male mating approaches of each of the species (Figure 3). The first PC is positively correlated with *A. biguttatus* approaches ( $r = 0.812$ ) and time ( $r = 0.844$ ), but negatively correlated with *A. angustulus* ( $r = -0.610$ ) and *A. sulcicollis* ( $r = -0.569$ ) approaches. All of these correlations are highly significant ( $P < 0.001$ ). This first PC explains 52% of the variation in these variables. The next PC explains 25% of the variation in the data. It is characterized by a positive correlation with *A. sulcicollis* approaches ( $r = 0.726$ ,  $P < 0.001$ ) and a negative correlation with *A. angustulus* approaches ( $r = -0.680$ ,  $P < 0.001$ ).

### Male choices of pinned females

Male *A. angustulus* usually made indirect approaches toward models onto the leaf surfaces within 2 cm of the models, with the exception of two direct paratrooper landings onto *A. angustulus* models. Whether walking toward the model after an indirect approach or accomplishing a

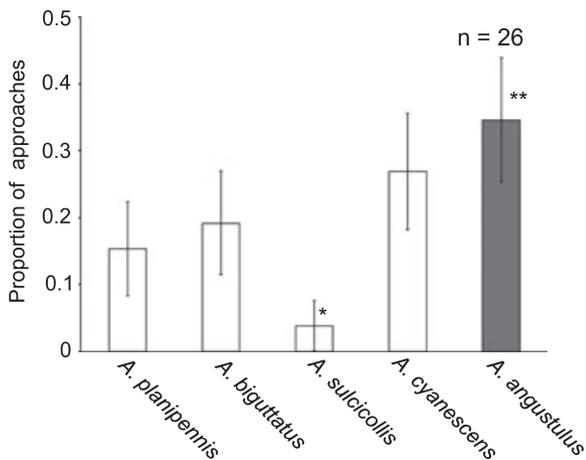


**Figure 3** Principle component (PC) scores for each of the 34 observation periods with respect to the first two PCs. Labels for *Agrilus biguttatus* and time are included to indicate a significant positive correlation between these factors and the first PC. The numbers of *Agrilus sulcicollis* and *Agrilus angustulus* observed per time period are negatively associated with the first PC, whereas they are positively and negatively correlated, respectively, with the second PC. Further details of this analysis are included in the text.

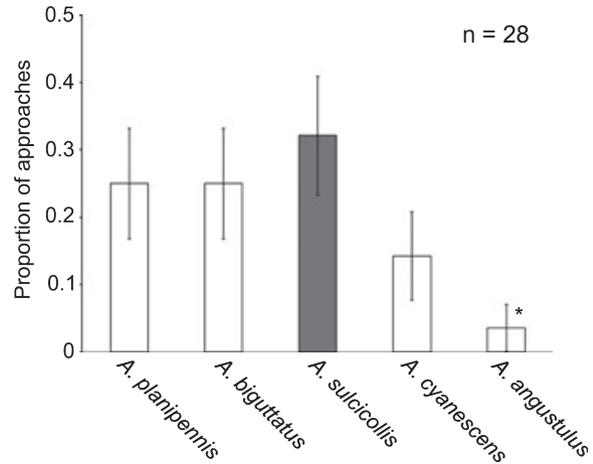
direct approach, males left after <1 s of contact. All of the five models attracted *A. angustulus* at least one time (Figure 4). However, conspecific models were approached most often (nine of 26 times). This proportion was significantly different (Fisher's exact test:  $P = 0.04$ ) from the proportion approaching *A. sulcicollis* (one of 26), but not from the proportions selecting other models. The interactions of model choice with additional factors such as the observation period, or the previous choice, were not statistically significant ( $P > 0.05$ ) if included in the model, and thus, these effects were not retained.

Approaches of male *A. sulcicollis* to models involved direct paratrooper descents. These males also quickly departed after <1 s of contact. *Agrilus sulcicollis* males performed these paratrooper approaches on all of the five models (Figure 5), but conspecific models were approached most often (nine of 28 times). This proportion was significantly different from that approaching *A. angustulus* (one of 28; Fisher's exact test:  $P = 0.04$ ), but was not significantly different from the proportion landing on other models ( $P > 0.05$ ). Again, the interactions of additional factors such as the observation period, or the previous choice, were not statistically significant ( $P > 0.05$ ) and those factors were not considered in the final model.

Male *A. biguttatus* flew directly onto the models from 1 m above. On both *A. planipennis* and *A. biguttatus* models, they often stayed for prolonged periods, attempting to copulate. During these copulation events, there were six



**Figure 4** Mean ( $\pm$  SE) proportion of male *Agrilus angustulus* flying within 2 cm of each of the five models, as calculated from the log linear model. Individual contrasts were made comparing the proportion landing on *Agrilus sulcicollis* to those landing on the other models. \*Significantly different proportion of approaches from *A. angustulus* (Fisher's exact test:  $P < 0.05$ ). \*\*Two of the nine approaches to *A. angustulus* were direct paratrooper landings.

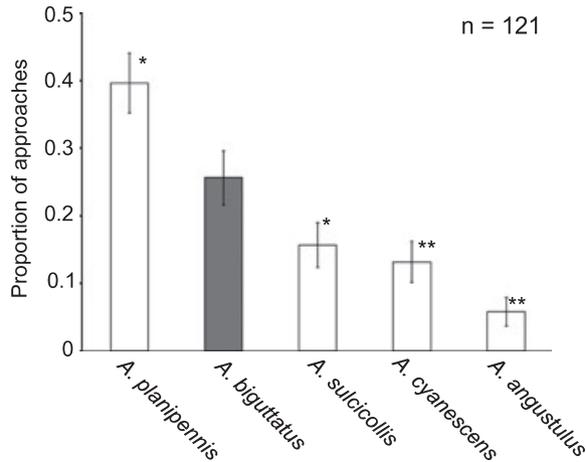


**Figure 5** Mean ( $\pm$  SE) proportion of male *Agrilus sulcicollis* paratrooper approaches to each of the five models, as calculated from the log linear model. Individual contrasts were made comparing the proportion landing on *A. sulcicollis* to those landing on the other models. \*Significantly different proportion of paratrooper approaches from *A. sulcicollis* (Fisher's exact test:  $P < 0.05$ ).

occasions when an additional male mounted a male that was already on a model. Only two additional paratrooper approaches were observed toward other female models when another *A. biguttatus* was already mounting one of the models. Neither of the above types of observations was included in the statistical analysis. In the 121 unbiased cases, males of *A. biguttatus* aerially approached every model type (Figure 6), but *A. planipennis* was approached most often. This proportion was marginally greater than that approaching the conspecific model (Fisher's exact test:  $P = 0.06$ ). Compared with the proportion descending onto *A. biguttatus*, the proportion choosing *A. sulcicollis* was marginally smaller ( $P = 0.09$ ), and the proportions choosing *A. cyanescens* or *A. angustulus* were significantly lower ( $P = 0.03$  and  $0.0004$ , respectively). The interactions of additional factors such as the unique observation period, or the previous choice, were not statistically significant ( $P > 0.05$ ) and thus not included in the model of *A. biguttatus* preference.

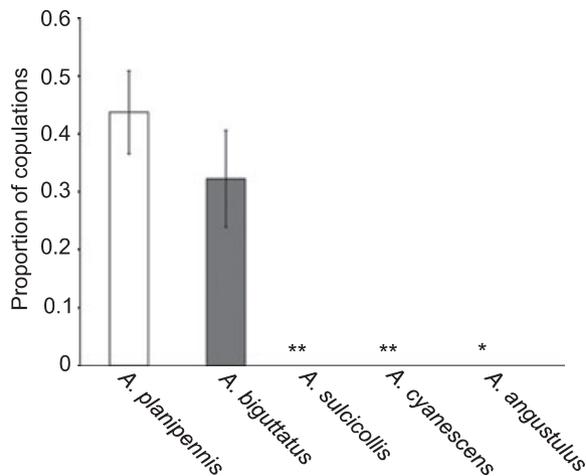
#### Copulation behavior

The only copulation behavior observed was of *A. biguttatus* males with either *A. planipennis* or *A. biguttatus* models (Figure 7). Together, the total duration of such observed copulation and guarding events accounted for 28% of the cumulative observation time in all periods when *A. biguttatus* was active. The proportion of paratrooper approaches leading to copulation was higher in *A. planipennis*, but this difference was not significant when using Fisher's exact test to compare the proportions.



**Figure 6** Mean ( $\pm$  SE) proportion of male *Agrilus biguttatus* paratrooper approaches to each of the five models, as calculated from the log linear model. Individual contrasts were made comparing the proportion landing on *A. biguttatus* to those landing on the other models. \*Marginally different proportion of paratrooper approaches from *A. biguttatus* (Fisher's exact test:  $P < 0.10$ ). \*\*Significantly different proportion of paratrooper approaches from *A. biguttatus* ( $P < 0.05$ ).

Comparison of the proportions of copulations per paratrooper landing observed on *A. biguttatus* to those on *A. sulcicollis* and *A. cyanescens* showed significant differences (Fisher's exact test:  $P = 0.004$  and  $0.009$ , respec-



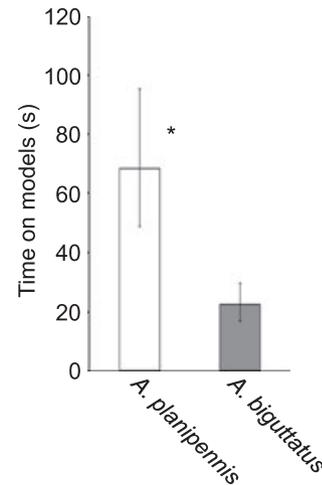
**Figure 7** Mean ( $\pm$  SE) proportion of male *Agrilus biguttatus* paratrooper approaches that led to copulation and mate guarding. Proportions were out of 27, 21, 19, 16, and 7 paratrooper events recorded toward *Agrilus planipennis*, *A. biguttatus*, *Agrilus sulcicollis*, *Agrilus cyanescens*, and *Agrilus angustulus*, respectively. \*Marginally different proportion of copulations observed vs. *A. biguttatus* (Fisher's exact test:  $P < 0.10$ ). \*\*Significantly different proportion of copulations observed vs. *A. biguttatus* ( $P < 0.05$ ).

tively). There is a marginal difference when comparing the copulation per approach proportion toward *A. biguttatus* vs. that toward *A. angustulus* ( $P = 0.09$ ). However, because there were zero mating attempts in only seven paratrooper approaches to *A. angustulus*, there was no power to detect a greater level of significance.

When *A. biguttatus* chose to remain on the models, they immediately initiated copulation, which was usually associated with wing fanning, lasting up to approximately 5 s. If the beetles stayed on top of the models for several minutes, there were often multiple bouts of copulation and wing fanning between periods of passive contact. Males lingered on the *A. planipennis* models for a significantly longer time than those on the *A. biguttatus* models (Figure 8). The maximum amount of time observed on an *A. planipennis* model was 17 min 12 s, and the longest duration spent on an *A. biguttatus* model was 1 min 40 s.

## Discussion

The three European buprestid species observed at this site all made visually mediated approaches toward dead, pinned beetle models. These field observations depict visual mate location behavior similar to that which was first described for *A. planipennis* and also observed in *A. cyanescens* and *A. subcinctus* (Lelito et al., 2007, 2009). Each of these cases demonstrates that such male visual location of females feeding on foliage is a critical mate-finding



**Figure 8** Mean ( $\pm$  SE) time spent on *Agrilus planipennis* and *Agrilus biguttatus* female models after paratrooper approaches that led to copulation and mate guarding. There were 21 and 10 such events on the two species models, respectively. Asymmetric error bars reflect standard error range calculated for the log-transformed data, which were used for ANOVA comparison of time on the two types of models (\* $P = 0.048$ ).

behavior. Because the attraction observed involves flight above the models, followed by a direct descent onto one of the available closely spaced models, it is clear that any female-emitted pheromone could not be involved in tracking the female during the execution of this particular behavior.

These observations of substantial cross-attraction among the oak-feeding species are similar to those in other observational studies of buprestids. Traps consisting of visual lures of *A. planipennis* and sticky material painted on the surrounding leaf captured the non-target species *A. cyanescens* and *Agrilus subcinctus* Gory (Lelito et al., 2008). Additionally, *A. cyanescens* males have been observed approaching and attempting to copulate with heterospecific lures, such as *A. planipennis*, and the non-buprestid tiger beetle, *C. sexguttata* (Lelito et al., 2011). Thus, it appears that a broadly tuned visual behavioral template for mate attraction may be common in buprestids.

Although odors emitted from the models could not have biased the flight approaches by wild males, it is still possible that each of these species uses pheromone signals to provide indications of promising locations for mate foraging. There is evidence of a beetle-produced attractant that plays such a role in *A. planipennis* (Bartelt et al., 2007; PJ Silk, pers. comm.). Thus, some of the behavioral differences between the species might be considered from this perspective. *Agrilus biguttatus* were most active and aggressive in approaching models, despite otherwise being rarely observed at the field site. Conversely, *A. angustulus* were pervasive in the foliage, but the males made relatively few mating approaches to our models. Perhaps such a pattern exists because mate foraging in *A. angustulus* is more strongly influenced by a pheromone signal that is used to locate conspecifics at favorable breeding sites. Furthermore, the indirect approach of *A. angustulus* whereby it lands on the leaf 1–2 cm from the model would seemingly make the use of a short-range pheromone a more useful part of the courtship process.

*Agrilus biguttatus* was active later in the afternoon than the two smaller species. Such temporal variation in mating activity may help prevent potential cross-species mating attempts between *A. biguttatus* and the other two sympatric species. Also, *A. biguttatus* approached the various models at a frequency that was correlated with their sizes. Thus, *A. biguttatus* may avoid cross-species mating attempts with these species because of a preference for larger mates during their in-flight approach. Although there was no evidence of temporal isolation between *A. sulcicollis* and *A. angustulus*, there was an indication of discrimination in their visual mate-finding mechanisms. The two species were both less likely to descend onto a

model of the opposite species than they were onto a conspecific model, whereas no such discrimination against the other species existed. The limited number of models used to assess the preferences of *A. sulcicollis* and *A. angustulus* at this location caution against presuming that such a mate-avoidance pattern is a definitive trait of both species. However, this pattern is intriguing given the broad cross-specific attraction otherwise observed in this study, coupled with previous studies on other *Agrilus* spp. that demonstrate that visual selection of conspecific models is not generally influenced by characters such as gender or by washing away cuticular surface chemicals (Lelito et al., 2008, 2011). For *A. cyanescens*, even heterospecific models can be as attractive as conspecific models (Lelito et al., 2011), making it seem in comparison unusual that species as similar as *A. sulcicollis* and *A. angustulus* would show any such visual discrimination. Thus, further exploration of the role of these visually mediated mating flights in facilitating the reproduction isolation of these species is warranted.

This pattern also illustrates the current poor understanding of how the visual mating signals are produced and perceived by buprestids. The blue coloration, often exhibited in *A. sulcicollis* and present in all of our models, might cause the relative aversion displayed by *A. angustulus* males to *A. sulcicollis* models. However, the more frequently chosen *A. cyanescens* models also had a similar blue iridescence. Moreover, insect species are known to employ visual mating signals undetectable by predators or humans. For example, the ultraviolet reflecting sheen emanating from the wings of male *Colias* butterflies provides species- and sex-specific mating avoidance signals (Silberglied & Taylor, 1973; Silberglied, 1979). It should also be noted that although the models of the native oak-feeding species had all been killed recently and handled in an identical manner, the specimens of *A. planipennis* and *A. cyanescens* were considerably older. The aging of the specimens may have introduced an unknown bias in the visually based mate choice behavior with respect to these non-oak-feeding models.

Our data clearly showed that *A. biguttatus* was the only species engaging in copulatory behavior with the models and that the only models eliciting such behavior were *A. planipennis* and *A. biguttatus*. The absence of copulation attempts directed toward the smaller models may have been influenced by the use of pins to fasten the models to the leaves. On the smaller models, the larger *A. biguttatus* was likely to encounter the pin while attempting to mount the female. Such an effect of the pin may also explain why the larger *A. planipennis* was mounted longer than *A. biguttatus*. Regardless of the possible bias caused by the pins, it is noteworthy that *A. biguttatus* copulated

with *A. planipennis* for such long durations. Previous studies show that copulation duration in *A. planipennis* is mediated by cuticular hydrocarbon signals, which encourage longer contact with females and shorter contacts with males (Lelito et al., 2009; Silk et al., 2009). Although this experiment did not test whether contact pheromones from the cuticle are necessary to elicit copulation by *A. biguttatus*, the hydrocarbon pheromone components that would presumably have been present on the *A. planipennis* models showed no evidence of deterring copulation by *A. biguttatus*. The duration of these copulatory mounts by *A. biguttatus* on *A. planipennis* was considerably longer than those described for conspecific mounts by *A. planipennis* (Lelito et al., 2007, 2009). Thus, with respect to the attributes of mating behavior we observed, there is no evidence for any reproductive isolation-producing mate-selection behaviors involving *A. biguttatus* males and *A. planipennis* females, which are allopatric species feeding on different host plants.

Overall, our results suggest both promise and caution for attempts to design and employ visually mediated traps for multiple buprestid beetle species. There are very high levels of cross-attraction among species, indicating a common behavioral template for visual mate attraction. However, because these sympatric species may be able to avoid certain heterospecific models, it is also possible that an incorrect calibration of the visual signal for such a trap might repel certain species. In this context, the results of our study may be particularly instructive in future attempts to design effective visual lure-based traps for the detection and monitoring of these oak-feeding buprestid species that could threaten North American oak forests and potentially other buprestid species that share this visual mate-finding behavior.

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