

RESEARCH ARTICLE

Differences in spectral selectivity between stages of visually guided mating approaches in a buprestid beetle

Michael J. Domingue^{1,*}, Jonathan P. Lelito², Andrew J. Myrick¹, György Csóka³, Levente Szöcs³, Zoltan Imrei⁴ and Thomas C. Baker¹

ABSTRACT

Spectral mating preferences were examined in male *Agrilus angustulus* (Buprestidae: Coleoptera), a member of a taxon known for its high species diversity and striking metallic coloration. The spectral emission profile of a typical *A. angustulus* female displays low chroma, broadly overlapping that of the green oak leaves they feed and rest upon, while also including longer wavelengths. To pinpoint behaviorally significant spectral regions for *A. angustulus* males during mate selection, we observed their field approaches to females of five *Agrilus planipennis* color morphs that have greater chroma than the normal conspecific female targets. *Agrilus angustulus* males would initially fly equally frequently toward any of the three longest wavelength morphs (green, copper and red) whose spectral emission profiles all overlap that of typical *A. angustulus* females. However, they usually only completed approaches toward the two longest wavelength morphs, but not the green morphs. Thus, spectral preference influenced mate selection by *A. angustulus* males, and their discrimination of suitable targets became greater as these targets were approached. This increasing spectral discrimination when approaching targets may have evolved to allow female emissions to remain somewhat cryptic, while also being visible to conspecifics as distinct from the background vegetation and heterospecific competitors.

KEY WORDS: Buprestidae, Color vision, Crypsis, Flight, Species recognition

INTRODUCTION

For arthropods, there have been extensive studies regarding the role of color preferences with respect to flower selection (Johnson and Midgley, 2001; Lunau et al., 2006; Yaku et al., 2007; Martinez-Harms et al., 2012; McCall et al., 2013), prey location (Taylor et al., 2014) and the efficacy of traps for monitoring and detection of pests (Francese et al., 2010; Gadi and Reddy, 2014). However, documentation of color preferences in mate selection has been less commonly described (Silberglie and Taylor, 1978; Kemp and Rutowski, 2011; Sánchez-Guillén et al., 2013). Male buprestid beetles (Coleoptera) perform visually guided mating flights toward females (Gwynne and Rentz, 1983; Lelito et al., 2007, 2011;

Domingue et al., 2014), making them promising candidates for the exploration of the mechanisms of color selection in beetles. The Buprestidae is a highly diverse family of 15,000 species (Bellamy, 2008), whose members are known for their dazzling arrays of metallic coloration patterns. Here, we examined more closely the role of color in mate-finding behaviors of an arboreal buprestid species, *Agrilus angustulus* (Illiger 1803). In addition to determining whether color preferences exist in their stereotypical mate-finding flights, we also explored the questions of whether such preferences might change during these brief flights, and what ecological factors might provide the selective pressures shaping these behaviors.

The stereotypical mate-location behavior in arboreal *Agrilus* buprestids involves a visual response of flying males toward females resting on the exposed upper surfaces of sunlit leaves. This behavior is characterized by the male rapidly descending from up to a meter above the female to ‘pounce’ directly onto her. The repeatability of the visually guided mating behavior was first documented in detail for *Agrilus planipennis* Fairmaire 1888 males approaching dead pinned specimens (Lelito et al., 2007, 2009). Similar behavior was later determined to exist for other arboreal *Agrilus* species (Domingue et al., 2011; Lelito et al., 2011). Many such arboreal buprestids cryptically reflect light at wavelengths similar to the leaves they rest upon, while also reflecting some light at longer or shorter wavelengths (Muskovits and Hegyessy, 2002; Bellamy, 2008). It was further noted in studies involving the mating behaviors of multiple *Agrilus* species that despite coloration and size differences, male pouncing behavior could also often occur toward heterospecific specimens (Domingue et al., 2011, 2013, 2014; Lelito et al., 2011).

Here, further insight into color discrimination and preference in *Agrilus* was obtained by directly observing male mating approaches to females with varied spectral emission profiles. Five female color morphs of *A. planipennis*, differing from each other in their peak spectral reflectance, were pinned to the leaves of European oaks in the field to assess the preferences of local *A. angustulus* males. The *A. planipennis* color morphs have greater chroma than a typical *A. angustulus* female target, whose spectral emission profile spans that of three of these morphs. This experiment thus allowed us to determine which particular portions of the spectrum emitted by *A. angustulus* females are most attractive in eliciting male mating responses, both at the initiation and at the completion of such stereotypical mating flights.

MATERIALS AND METHODS

Agrilus planipennis color morphs

All *A. planipennis* female color morphs were obtained from a large-scale rearing facility in Brighton, MI, USA, run by the USDA Animal and Plant Health Inspection Service. The facility continuously rears *A. planipennis* using natural host plant

¹The Pennsylvania State University, Department of Entomology, University Park, PA 16802, USA. ²United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Brighton, MI 48116, USA. ³NARIC Forest Research Institute, Department of Forest Protection, Mátrafüred 3232, Hungary. ⁴Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest 1022, Hungary.

*Author for correspondence (mjd29@psu.edu)

 M.J.D., 0000-0002-3055-3533

material. Most individuals of the species are observed to be green, but rarely phenotypes emitting longer (red) or shorter (blue) wavelengths have been observed (<1% of all beetles). Pairs of similar rare phenotypes were mated to each other in an attempt to produce true-breeding lines of these differently colored beetles. Lines were obtained in which at least half the offspring had mutant phenotypes, but a detailed genetic analysis of these lines will not be presented here. Within the short-wavelength lines, two visibly distinct phenotypes, blue and violet, were noticed (Fig. 1A). Likewise, within the long-wavelength lines there were often two other discrete phenotypes, copper and red (Fig. 1A). Sample sets of females of each color morph were set aside for behavioral experiments. The females were killed by freezing overnight and pinned through the anterior portion of the pronotum. The mean (\pm s.e.m.) lengths of the beetles in these samples were not substantially different across color morphs (violet: 11.60 ± 0.19 mm, blue: 11.73 ± 0.37 mm, green: 12.14 ± 0.09 mm, copper: 11.55 ± 0.05 mm, red: 11.96 ± 0.19 mm). Similarly, the mean (\pm s.e.m.) pronotum widths deviated little among morphs (violet: 2.90 ± 0.04 mm, blue: 2.83 ± 0.06 mm, green: 2.91 ± 0.03 mm, copper: 2.85 ± 0.05 mm, red: 2.87 ± 0.05 mm). A single specimen of each color morph was also set aside for spectral reflectance analysis. Because such analysis required a destructive preparation, and the number of color morphs obtained was limited, we did not perform measurements from all specimens from all lines. Destroying the samples to perform such measurements would preclude the possibility of future behavioral experiments involving these rare morphs and other *Agrilus* species.

Reflectance spectrophotometry

The following samples were assayed for relative wavelength emission from 300 to 850 nm: one female of each of the four rare *A. planipennis* color morphs (violet, blue, copper and red), nine wild-type (green) *A. planipennis* females, 27 male and 25 female field-collected *A. angustulus*, and single female specimens of two other common European oak buprestids, *Agrilus sulcicollis* Lacordaire and *Agrilus biguttatus* Fabricius. Reflectance spectra were also recorded from a single oak leaf (*Quercus alba* L.) and an ash leaf (*Fraxinus pennsylvanica* Marshall). The leaf measurements

were not intended to be characteristic of the spectral reflectance patterns of the leaves used in the experiment, but rather to provide the reader with a visualization of where in the spectrum peak reflectance due to chlorophyll pigmentation typically occurs in comparison to the beetles. Measurements were performed using a Lambda 950 photospectrometer (PerkinElmer, Bridgeville, PA, USA) with a 150 mm integrating sphere equipped with a microfocus lens and mechanical iris to establish a beam size of 2 mm, which had an angle of incidence of 0 deg. All sample spectra were referenced to a Spectralon™ (Labsphere, Inc., North Sutton, NH, USA) reflectance standard. Specimens were prepared using elytra from dead specimens that were less than 1 year old and maintained at room temperature in closed containers to prevent degradation that might influence coloration. The beam illuminating the specimens was variably larger than the elytra of the *A. angustulus* specimens. Thus, accurate comparative measurements of total reflectance of this species were not possible. The wavelength of peak reflectance from each trace was assessed after smoothing using cubic spline interpolation. The smoothed traces were compared with the original data to ensure that the shape and position of the peaks did not shift. Although this procedure was used to provide the most accurate estimate of peak reflectance, unaltered spectral traces are used in all displays of the data.

Field site

Observations and collections were performed in a mixed oak forest site near Mátrafüred, Hungary ($47^{\circ}50'17''N$, $19^{\circ}59'50''E$). Within this forest, logging is active yearly, and *Agrilus* beetles are readily found on leaves exposed to direct sunlight on the south-facing tree branches above log piles (Domingue et al., 2011, 2013, 2014). All observations occurred on two neighboring sessile oaks, *Quercus petraea* Liebl. 1784, each with foliage 2–3 m from the ground. The south-facing branches of these trees received direct sunlight between 11:30 h and 15:00 h. Beetles were observed by a researcher standing on a 1.5 m-tall moveable ladder to raise the observer to eye level with the lowest branches. The observational periods occurred between 18 and 28 June 2014.

Field observations

An experiment was devised to assess the spectral preferences of male *A. angustulus* for female *A. planipennis* color morphs. In addition to the availability of the morphs, one of the advantages of this particular study is that the use of female *A. planipennis* morphs, which have narrower spectra (high chroma) than *A. angustulus* females, allows us to decompose which narrower ranges of wavelengths are most important for influencing behavior. One each of five pinned dead female *A. planipennis* color morphs, violet, blue, green, copper and red (Fig. 1A), were placed approximately 10 cm apart on neighboring leaves (Fig. S1). There were at least four specimens of each color morph, which were exchanged daily for others of the same morph. All specimens were pinned through the thorax, which allowed them to be affixed to the leaves and rearranged between 10 min observational replications, as described in previous experiments (Domingue et al., 2011, 2014), to prevent positional bias.

Previous video-recorded observations of *A. planipennis* and *A. biguttatus* males (Lelito et al., 2007; Domingue et al., 2014) showed initial flights of up to 1 m lasting as long as 1 s, often followed by a brief 100–200 ms hovering period within approximately 25 cm of a pinned dead beetle targeted for mating or a synthetic decoy. After hovering, the males usually flew toward one of the presented mating targets in a flight with a duration of less than 200 ms. They

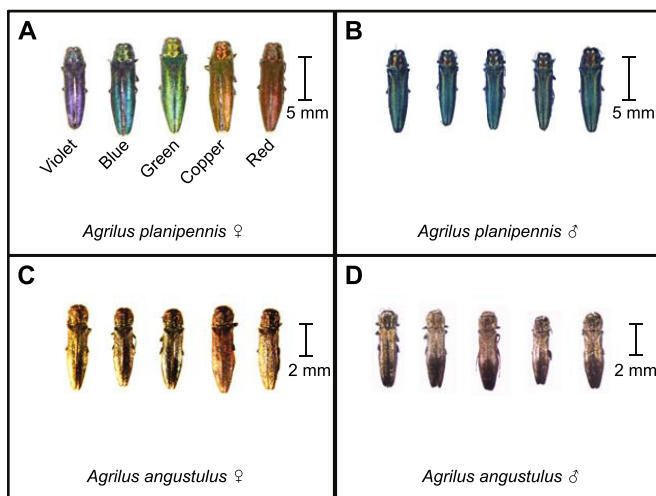


Fig. 1. Representative specimens of *Agrilus planipennis* and *Agrilus angustulus*. Depictions include five *A. planipennis* female color morphs (A), five green-morph *A. planipennis* males (B), five field-collected *A. angustulus* females (C) and five field-collected *A. angustulus* males (D).

sometimes landed directly upon the mating target, but other times upon the neighboring leaf surfaces. In this experiment, we observed similar flight trajectories and noted the targets of the males, which became apparent subsequent to this brief hovering event. It was then observed whether the male landed directly on a pinned beetle or diverted onto the neighboring leaf surface.

Previous experiments showed a wide range in the likelihood that a male beetle would land upon a target. For example, *A. biguttatus* males often flew toward crude plastic decoys, but diverted away in 93% of subsequent approaches (Domingue et al., 2014). However, this deflective behavior was observed for only 26% of approaches to realistic decoys and 4% of approaches to real dead females. Likewise, the small species *A. angustulus*, which usually flew toward other congeneric species, including wild-type green *A. planipennis* specimens, always diverted away to land at least 1 cm away from the target (Domingue et al., 2011). This failure to land directly on the targets also occurred when *A. angustulus* males approached pinned conspecific females, but for this species the head of the pin is very large relative to the specimen, and may have perhaps influenced the behavior.

Most of the recorded observations in this experiment were of male *A. angustulus*, which is highly ubiquitous at this site (Domingue et al., 2013), although there were a few approaches by males of *A. biguttatus*, *A. sulcicollis* and another unidentified *Agrilus* species, all with obviously different coloration from that of male *A. angustulus*. Prolonged mounting and attempts to copulate with the much larger female *A. planipennis* specimens by *A. angustulus* males never occurred, regardless of the female color morph they landed upon. Species and sex recognition after contact have not been studied for *A. angustulus*, but are influenced by contact chemoreception of cuticular hydrocarbons in other *Agrilus* species (Lelito et al., 2008, 2011). Whether or not the male insect landed upon the female color morph, it was visually followed to observe the location where it landed, which was usually upon a neighboring leaf. It was thus visually identified as having a size and coloration within the range expected for *A. angustulus*. To further confirm the ability of the observer to correctly assign species identity of the observed wild males, and to provide specimens for spectral reflectance measurements, a subsample of more than 100 presumed *A. angustulus* males and females were collected and identified in the lab according to traits listed by Muskovits and Hegyessy (2002). An additional 20 presumed *A. angustulus* were collected directly after observing the mating approaches toward the female color morphs. All 20 of these specimens were positively identified as *A. angustulus* males.

Statistical analyses of behavioral data

Agrilus angustulus was the only species for which enough observations were made to permit statistical analyses. For comparing the number of initial approaches made in the field by *A. angustulus* males to the female *A. planipennis* color morphs, a log linear model was fitted to the data using the Proc CATMOD feature of SAS v.9.2 (SAS Institute, Cary, NC, USA). This model also allowed assessment of the significance of additional experimental factors, such as each unique rotation of the samples, and the date when the experiment was performed. Such additional factors were not retained in the final model because they were never significant at $\alpha=0.05$. They also often contributed to the model not converging if retained. Contrasts were evaluated to compare the preference between each possible combination of pairs of color morphs. We used Barnard's exact test with a Bonferroni correction for experiment-wise error for

comparing the proportion of initial approaches that led to direct landings on each of the morphs.

RESULTS

Reflectance spectra

The mean (\pm s.e.m.) wavelength of maximum reflectance of *A. angustulus* specimens was 562.1 ± 3.1 nm (range: 536–603 nm) for 27 males and 592.0 ± 4.8 nm (range: 542–650 nm) for 25 females. These means are significantly different using Satterthwaite's approximate *t*-test ($t=5.23$, d.f.=41.3, $P<0.0001$). However, because the range of peak reflectance overlaps substantially, individual specimens cannot be visually distinguished by the human observer as male or female based upon this character. The median trace with respect to peak wavelength reflectance for both sexes is presented in Fig. 2A, with maxima at 561 nm for males and 592 nm for females. The peaks in the reflectance spectra for this species were quite broad, with elevated values throughout much of the emitted spectrum. Most of the *A. angustulus* specimens thus appeared green, yellow or orange when observed under very bright light. Female specimens are shown in Fig. 1C, which tended to appear yellow, orange and red, more so than males (Fig. 1D).

As expected, each representative *A. planipennis* color morph had distinct reflectance peaks as follows: violet, 425 nm; blue, 454 nm; wild-type green, 532 nm; copper, 610 nm; and red, 694 nm (Fig. 3A). The green morph spectrum depicts the median peak reflectance among nine samples, which had a mean (\pm s.e.m.) peak reflectance of 533.6 ± 2.5 nm. Spectra for a single typical *A. sulcicollis* female had a maximum reflectance at 477 nm, while an *A. biguttatus* female had a peak reflectance similar to *A. angustulus* at 605 nm (Fig. 2B). The oak and ash leaves tested had mid-wavelength peaks at 552 nm, in addition to sharply rising reflectance from 700 to 850 nm (Fig. 2C).

Field behavioral assay

In total, 71 approaches by *A. angustulus* males toward one of the distinct color morphs were observed after the brief hovering phase at approximately 25 cm above the targets. There were only a few cases in which no target was selected and the beetle flew away without making a clear choice toward a target. These observations were not included in the analysis. Thirty-nine of these approaches led to the wild males landing directly on the pinned *A. planipennis* color morph, whereas 32 resulted in the beetle landing on the leaf surface neighboring the *A. planipennis* color morph. In each case, it could clearly be discerned which color morph was approached and whether the approach was completed.

There were significant differences among the color morphs with respect to the number of wild *A. angustulus* males approaching them after the hovering phase ($\chi^2=15.99$, d.f.=4, $P=0.003$). The greatest proportion of the 71 responses occurred to the copper morph ($N=24$, 34%), followed closely by the green ($N=19$, 27%) and red ($N=16$, 23%) morphs (Fig. 3C), with percentages that are not significantly different. The fewest flying approaches ($N=4$, 6%) were toward the violet morph, the one with the shortest wavelengths. Males flew toward the violet-colored beetles significantly less often than all the other morphs with the exception of blue ($N=8$, 11%).

The next variable measured was the conditional probability of landing on each color morph, given a flying approach. Longer wavelength morphs were more successful at eliciting the completion of the flying approaches onto the pinned beetles (Fig. 3D). For both the red and the copper morphs, approximately 80% of the approaching *A. angustulus* males landed directly upon the targets to complete the in-flight approach (19 of 24 and 13 of 16 approaches

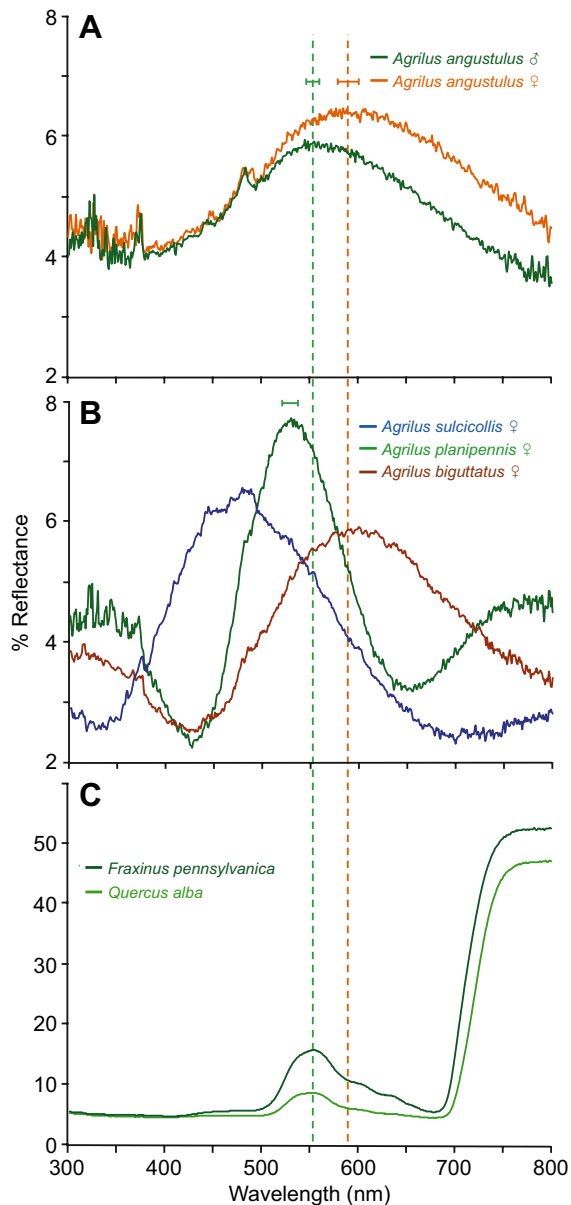


Fig. 2. Spectral emission profiles of insect and foliar targets potentially viewed by arboreal *Agrilus* species. Specimens include male and female *A. angustulus* (A), females of *A. sulcicollis* (blue), *A. planipennis* (green) and *A. biguttatus* (brown) (B), and *Fraxinus pennsylvanica* and *Quercus alba* leaves (C), which respectively are common hosts of *A. planipennis* and all of the other *Agrilus* species. Where error bars are present for *A. angustulus* males ($N=27$), *A. angustulus* females ($N=25$) and *A. planipennis* females ($N=9$), the spectra of median peak reflectance are displayed with a ± 2 s.e.m. bar centered at mean peak reflectance. Vertical dashed lines provide references for the peak reflectances of leaf and *A. angustulus* specimens.

were completed, respectively). The proportions of completed direct landing events onto the green (6 of 19, 32%), blue (1 of 8, 13%) and violet (0 of 4, 0%) morphs were all significantly lower than those to the copper and red morphs (Barnard's exact test, Bonferroni correction; $\alpha=0.05$).

DISCUSSION

Flights toward the mating targets were observed to begin at a distance of up to 1 m, but given the small size of the eyes of these beetles, it is not likely that image formation was possible at this

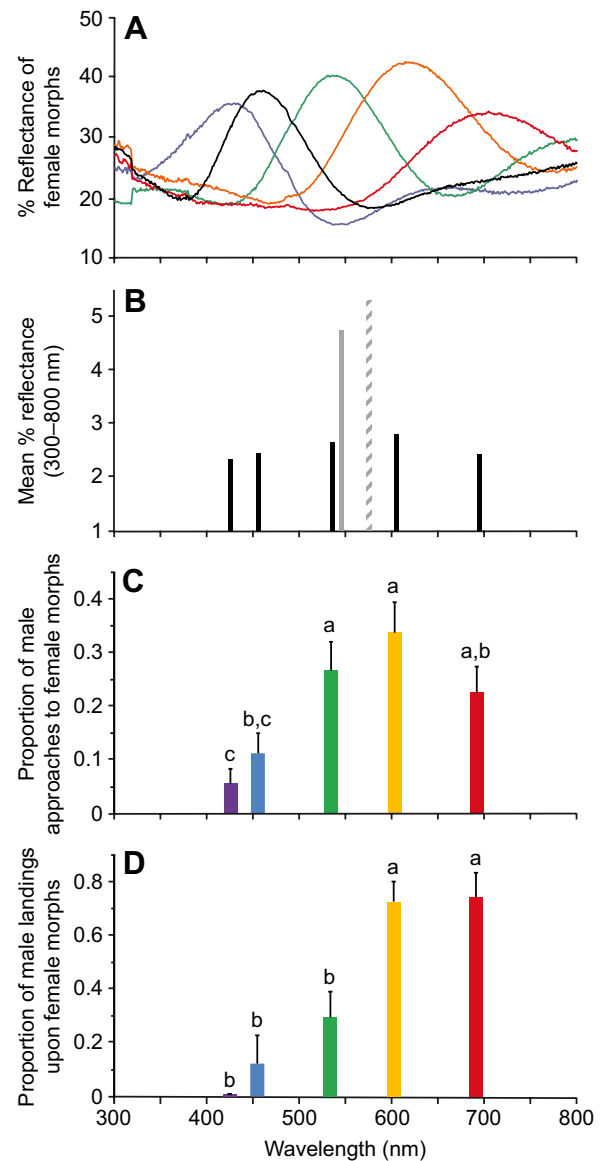


Fig. 3. Spectral emission and response profiles for *A. planipennis* color morphs presented to *A. angustulus* males. (A) Reflectance of *A. planipennis* female color morphs. (B) Mean reflectance from 300 to 800 nm for each *A. planipennis* female color morph (black), and the *A. angustulus* male (gray) and female (striped) at their respective peak wavelengths. (C,D) Mean proportion (\pm s.e.m., $N=71$) of *A. angustulus* males (C) initially approaching each *A. planipennis* female color morph and (D) landing, given an initial response toward that color morph. Identical letters within panels indicate that responses did not significantly differ at $\alpha=0.05$ (χ^2 tests with Bonferroni correction for experiment-wise error).

distance (Hempel de Ibarra et al., 2014). Rather, it is more probable that the female target was detected because the insect was encountering strands of light scattered by the cuticle surface (Domingue et al., 2014), creating a high flicker-fusion frequency, which is known to be detectable by insect eyes (Miall, 1978). Furthermore, because the five color morphs were placed relatively close together on neighboring leaves, our experimental design could not have determined whether the color morphs differ in attraction at the approximately 1 m distance where movement toward the target was first observed. It is more likely that the wavelength-based selection of the target (Fig. 3C) began when the insect briefly hovered at approximately 25 cm above the specimens. While the

visual mechanisms for the color morph preference exhibited at this distance are not fully understood, it seems likely that wavelength specificity is a factor, with a preference for mid to high wavelengths. The violet and blue morphs were very rarely approached despite having mean reflectance across the 300–800 nm range that is similar in magnitude to that of the more frequently approached red morph (Fig. 3A,B).

Numerous examples of color vision exist in insects, and all insects studied have two or more photoreceptors of differing spectral sensitivity (reviewed in Kelber, 2001; Kelber et al., 2003). It is believed that the ancestral condition of insects is to have three opsins corresponding to ultraviolet (350 nm), short (440 nm) and long (530 nm) wavelengths, but the short wavelength opsin has not been found in Coleoptera (Briscoe and Chittka, 2001). Furthermore, of four Buprestidae species investigated, including *A. planipennis*, all displayed duplications of ultraviolet and long wavelength opsin genes (Lord et al., 2016). For *A. planipennis*, four peaks in electroretinogram recordings of males were noted at different points of the spectrum from 300 to 700 nm (Crook et al., 2009). Therefore, *A. angustulus* males likely also have some degree of spectral selectivity, which is compatible with color discrimination contributing toward the visually guided mating behaviors observed in this study. Most interestingly, wavelength-specific discrimination by males rapidly narrowed as they approached the targets. Flights were completed most frequently when proceeding toward the copper and red morphs, but not after the also common flights toward the green morphs (Fig. 3C,D). Thus, there also appears to be a temporally unfolding increasing preference for the longer wavelengths. The importance of chromatic perception in influencing this rapidly changing preference is supported by the high completion rate of flights toward the red morph, despite its lower reflective intensity in comparison to the green morph. The green morphs were usually not landed upon, despite the high frequency of flights toward them and the relatively strong intensity of the light emitted by the targets (Fig. 3B). The wavelength assessment by *Agrius* males thus involves a narrowing of preference by approximately 60–70 nm during the 100–200 ms duration of the final flight approach.

It was previously observed that *A. biguttatus* males became more selective about landing upon two different synthetic replicas that were both initially attractive (Domingue et al., 2014). In that case, they landed on nano-bioreplicated replicas that scatter light in a distinctive pattern similar to real beetles, but diverted away from similarly colored simpler replicas that instead emitted an untextured glow of light. Thus, *Agrius* seem generally selective about completing mating approaches, hinting at a potential costs to the behavior, such as an increase in predation risk, or wasted effort in heterospecific mating attempts. Sequentially unfolding changes in responsiveness to stimuli have also been documented for male *Ostrinia* moths, which experience a broadening of receptivity to female pheromone blends as they approach the odor source (Kárpáti et al., 2013). Thus, *Ostrinia* conversely would be expected to experience a greater cost for incorrectly initiating rather than completing mating approaches.

It has been noted in a study of pollinators that spectral sensitivities of opsins tend to be optimized to match not only the maxima of the visual target but also the inflection points surrounding the maxima (Shrestha et al., 2013). Although such spectral sensitivities are not known for *A. angustulus*, when further interpreting the functional significance of the narrowing spectral preferences in *A. angustulus*, it should also be considered that the *A. planipennis* morphs have much greater chroma than the typical *A. angustulus* female target.

The wavelengths emitted by the green, copper and red morphs all overlap substantially with those emitted by typical *A. angustulus* females (Figs 2A and 3A). Thus, it is more appropriate to interpret the shift in behavioral discrimination of the morphs in the context of how *A. angustulus* males can optimally track conspecific females. At further distances, detecting any of the wavelengths that are emitted at an elevated level by *A. angustulus* females may assist in locating the target. However, the males may be relying on emission of longer wavelengths to verify the species identity of *A. angustulus* females as they approach. Because of their greater chroma, the green *A. planipennis* morphs lack such longer wavelength emissions, but the copper and red morphs, like all *A. angustulus* females, will have substantial spectral emissions above 600 nm.

The ecological context of this chromatic discrimination in *A. angustulus* may be provided by competitive interactions with other buprestids, as well as selective pressures for a cryptic appearance that minimizes predation risk. For example, the sympatric oak-feeding competitor *A. sulcicollis* (Fig. 2), which is slightly larger than *A. angustulus*, is blue in coloration. It was previously noticed in field choice experiments that these two species rarely approached each other, despite both being highly attracted to specimens of other *Agrius* species (Domingue et al., 2011). Thus, avoidance of shorter wavelength spectral emissions and preference for longer wavelengths by *A. angustulus* may promote species recognition and help to avoid landing events upon *A. sulcicollis*. Furthermore, the narrowing of spectral preference as the target is approached suggests that there is less cost associated with flying toward other species than there is in landing upon them and attempting to initiate mating. While another sympatric oak-feeding species, *A. biguttatus*, has a spectral reflectance that is quite similar to that of *A. angustulus* (Fig. 2), it is much larger and has distinctive white spots on the elytra (Muskovits and Hegyessy, 2002). Thus, mistaken heterospecific mating attempts by *A. angustulus* toward *A. biguttatus* could be reduced by utilizing a non-spectral visual characteristic such as this.

There is little direct evidence of selection for crypsis in *Agrius*, but several observations make its strength as a substantial selective pressure seem plausible. First, many arboreal buprestids, including *A. angustulus*, have spectral emissions that overlap those of the leaves they rest upon, as shown in Fig. 2. Additionally, the specialist nesting wasp *Cerceris fumipennis* is presumed to locate adult buprestid prey visually (Rutledge et al., 2014), and birds have been observed taking these beetles from green sticky traps in other experiments (M.J.D., unpublished observations). It is also noteworthy that male *A. angustulus* tend to have shorter-wavelength peak spectral emissions that more closely match those of leaves when compared with females. Peak female spectral emissions are on average 30 nm longer than those of conspecific males and the leaves they rest upon. Females may thus be experiencing counterselective pressures for mid wavelength emissions that promote crypsis and longer wavelengths that facilitate mate recognition. Trade-offs between natural selection for visual crypsis and sexual selection for mate recognition have also been described for a variety of other taxa (Barry et al., 2015; Fincke, 2015; Bossu and Near, 2015).

This study also contributes to accumulating evidence for a high degree of malleability in the visual signaling systems of Buprestidae. It has previously been noted that *A. sulcicollis* can display a range of coloration from blue to green (Muskovits and Hegyessy, 2002). This study shows a similar gradation of coloration from green to red in *A. angustulus*. Furthermore, the ability to select for more extreme color morph variants in the *A. planipennis* colony affirms a degree of heritable polymorphism that in other systems has

been associated with high speciation rates (Hugall and Stuart-Fox, 2012; McLean and Stuart-Fox, 2014) and particular speciation scenarios (Richards-Zawacki et al., 2012; Albertson et al., 2014). Finally, the spectral response preference of *A. angustulus* males was shown to include red targets (~694 nm), which have a chromatic content well outside the normal range of peak wavelength emission of conspecifics (542–650 nm). Such sensory biases can potentially be exploited to encourage evolutionary changes to signaling systems (Basolo, 1990; Boughman, 2002; Arnqvist, 2006).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

M.J.D. developed concepts, designed experiments, performed experiments and data analysis, and prepared the manuscript. J.P.L. designed experiments and performed experiments to obtain colour morphs. A.J.M. performed data analysis. G.C., L.S. and Z.I. developed concepts and assisted in insect location and experimentation. T.C.B. developed concepts and revised the manuscript.

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Data availability

All data are available through figshare: figshare.com/s/161e6cb4276511e5855e06ec4b8d1f61

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.137885.supplemental>

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