RESEARCH PAPER

The Influence of Age on Male Mate-Searching Behaviour in Thornbug Treehoppers

Paul A. De Luca & Reginald B. Cocroft

Division of Biological Sciences, University of Missouri, Columbia, MO, USA

Correspondence
Paul A. De Luca, Department of Biological Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, ON, Canada M1C 1A4.
E-mail: paul.deluca@utoronto.ca

Abstract

One prediction from life-history theory is that males should increase investment in reproductive effort as they age because the opportunity for future reproductive events declines. However, older males may not be able to increase their reproductive effort if condition declines with age. The effect of age-related changes in condition may be especially important for energetically costly activities such as moving within and between habitat patches while searching for mates. Although such searching is a component of many mating systems, the relationship between age and active mate searching has not been investigated. We investigated whether mate-searching effort increased with age in the thornbug treehopper, *Umbonia crassicornis* (Hemiptera: Membracidae). In this species, males search for females using a 'fly-call-walk' strategy consisting of three phases: (1) flying from one plant to another; (2) walking and signalling while on a plant; and (3) close-range courtship of encountered females. We measured several aspects of mate-searching behaviour over the month-long period of a male's reproductive lifetime. Over the relevant period of male sexual activity (19–33 d), male condition remained stable. However, older males (25–33 d) did not search more actively than younger males as predicted; instead, younger males (19 d) had greater plant-to-plant flight activity and found females faster. Within-plant walking rates and courtship duration did not differ among age classes. These results suggest that thornbug males may be investing so heavily in mate searching at younger ages that they are unable to increase investment in searching effort when they get older. As a result, older males are likely to be at a competitive disadvantage when active searching is required to locate sparsely distributed females.

Introduction

Life-history theory predicts that as males age they should increase investment in reproductive effort because their residual reproductive value (RRV) – the opportunity for future reproductive events – declines (Stearns 1992). Such investment is often manifested as age-related increases in the intensity, rate, or duration of a costly behaviour important for acquiring mates. Sexual traits showing the predicted positive relationship between RRV and reproductive effort include visual and acoustic mate advertisement signals (Bertram 2000; Miller & Brooks 2005; Garamszegi et al. 2007; Jacot et al. 2007; Bitton et al. 2008; Alonso et al. 2010), and courtship behaviour (Woodhead 1986; Höglund & Lundberg 1987; Conner 1989; Felton et al. 2006; Alonso et al. 2010). Recent studies, however, have shown that the physical condition of males is an important influence on the nature of the relationship between age and

reproductive effort. If condition is high in young males, they may invest so heavily in sexual display that they either die young or lose condition as they get older, in which case they may be physiologically unable to make further investments in reproductive effort (Hunt et al. 2004). On the other hand, younger males in high condition can also invest more in survival early on and then devote more resources towards increasing reproductive effort when they are older (Judge et al. 2008). Accordingly, whether older males increase reproductive effort depends greatly on how condition changes over the reproductive lifetime of an individual.

In many species, males engage in exploitation competition through signalling for mates and/or moving through the habitat searching for females (Parker 1978; Andersson 1994; Shuster & Wade 2003). Age-related increases in signalling effort are well documented and have been shown to be influenced greatly by a male's condition (Howard 1978; Zeigler & Stewart 1985; Hedrick 1986; Ritchie et al. 1995; Kokko 1997; Brooks & Kemp 2001; Gil et al. 2001; Fischer et al. 2004; Forstmeier et al. 2005; Garamszegi et al. 2007; Jacot et al. 2007; Judge et al. 2008). However, the combined effects of age and condition on active mate-searching behaviour have never been explored. This is surprising, considering that mate searching is an important component of mate acquisition in a number of species and has been extensively studied in many other respects (Woolbright et al. 1990; Forbes et al. 1996; Duvall & Schuett 1997; Sikkel 1998; Brown & Weatherhead 1999; Uy et al. 2001; Diaz & Thiel 2004; Dunn & Whittingham 2007; Kasumovic et al. 2007). If active mate searching varied with age and condition, there might exist different mating strategies between younger and older males (De Fraipont et al. 1993), which could have consequences for mating system evolution (Shuster & Wade 2003). Accordingly, some researchers have recently advocated the need for more experimental studies examining the extent to which life-history processes such as ageing and condition influence variation in the expression of male mating behaviours (e.g. Brooks & Kemp 2001; Bonsall & Mangel 2004; Hunt et al. 2004; Fischer et al. 2008).

Plant-dwelling insects provide an opportunity to examine the relationships between age, condition, and active mate searching because in many species, males conduct frequent plant-to-plant flying, combined with extensive walking within plants, to locate receptive females (Claridge 1985; DeWinter & Rollenhagen 1990; Hunt & Nault 1991; Claridge & de

Vrijer 1994; Hunt 1994; Cökl et al. 1999; Cocroft 2003; Demary et al. 2006). As with mate advertisement signalling, the intensity of mate searching may also be influenced by age-associated changes in condition. For example, if condition is stable with age, older males might increase their investment in mate-searching effort as predicted from life-history theory, resulting in greater levels of flight activity and within-plant walking behaviour, and thus increased female encounter rates. On the other hand, if condition declines with age, older males may be physiologically unable to make further investments in searching effort and might exhibit decreased searching activity, and thus fewer encountered females. However, if low condition older males could switch their reproductive effort to another activity that was not as energetically demanding, such as close-range courtship of encountered females, they may still be successful at acquiring mates (Woodhead 1986; Andersson 1994; Felton et al. 2006; Engqvist 2009). Additional time spent with one female, though, has an opportunity cost, taking time away from seeking other potential mates (Parker 1978; Jordan & Brooks 2010).

In the thornbug treehopper, *Umbonia crassicornis* (Hemiptera: Membracidae), individuals develop in aggregations of one or more family groups, from which males disperse before females (Wood 1974). Females mate only once, and do so under one of two conditions: some mate while still in their family aggregation, while others mate after dispersing (Wood & Dowell 1985). Although females that disperse before mating are sparsely distributed throughout their habitat, they represent a significant pool of mating opportunities for mate-searching males (Wood & Dowell 1985; Masters 1997). Accordingly, dispersed females are most likely to be encountered by males that are best at active searching. Males search for females using a 'fly-call-walk' strategy consisting of three phases: (1) flying from one plant to another; (2) walking and signalling while on a plant; and (3) close-range courtship of encountered females. Flying males will land on a plant and advertise for females using substrate-borne vibrational mating signals. Receptive females reply with a vibrational signal, but if no female replies are received the male usually leaves the plant and flies to another (Cocroft & McNett 2006; De Luca & Cocroft 2009). If a female reply is detected, the male and female engage in an ongoing duet that enables the male to locate the female. The male then engages in close-range courtship, which involves climbing onto the side of the female (Wood 1974; De Luca &

Cocroft 2008) while producing a series of vibrational signals and attempting genital contact (PA De Luca, own data). In addition to signalling to elicit a reply from receptive females, males will court any female that is encountered during searching, including sexually immature females that are still aggregated in their family group. Two previous studies with *U. crassicornis* found that aggregated pre-dispersal females mated more often with older (30 d) than younger (20 d) males, and furthermore, in these high-density aggregations older males also courted females at significantly greater rates (Wood & Dowell 1985; De Luca & Cocroft 2008). Whether older males also have a mating advantage with dispersed females is unknown, but this is likely to depend on whether older males can outperform younger males during active mate searching.

Here, we examine the relationships between age, condition, and active mate-searching behaviour to determine whether *U. crassicornis* males increase investment in mate-searching effort as they get older. For individual males, we measure changes in physical condition and characterize the three phases of mate searching (flight activity, within-plant walking, and courtship) weekly over the month-long period of their reproductive life span. To the extent that condition remains stable with age, we predict that older males will increase their mate-searching effort by exhibiting higher levels of flight and walking activity, enabling them to find females faster on a plant. However, if condition declines with age, we predict that older males will be unable to make additional investments in flying and walking behaviour and therefore will not be as active in mate searching, resulting in longer search times to find females. Under these circumstances, we further predict that older males will spend more time courting individual females when they are encountered, to compensate for their decreased searching activity.

Methods

Study Organism

Thornbug treehoppers range from Colombia to southern Florida, USA (Mead 1962). This species uses a variety of host plants, primarily in the Mimosaceae (McKamey & Deitz 1996). Females are monogamous and lay a single clutch of eggs in their lifetime (Wood 1974). Females care for their developing young by making feeding slits on the host plant stem, and by protecting them from predators (Cocroft 2002). Siblings eclose to adulthood within

2–3 d of one another and then remain aggregated for 15–20 d before dispersing from their natal plant (Wood & Dowell 1985; Masters 1997). Adult males and females reach sexual maturity at approximately 12 and 21 d, respectively (De Luca & Cocroft 2008). Males disperse earlier than females: by the time females reach sexual maturity, 25% of males and 50% of females remain in their natal aggregation (Wood & Dowell 1985). The life span of adult males in the field is unknown, but males reared in a predator-free greenhouse environment do not live beyond 50–60 d. Accordingly, the period of male sexual activity begins approximately 12 d after adult eclosion and ends 4–5 wk later (De Luca & Cocroft 2008).

Insect Maintenance

We used individuals in this study that were first-generation descendents of insects collected in and around Miami, FL, USA, in 2006. We collected nine family groups (20–40 individuals each) as late-instar nymphs or teneral adults that were still aggregated. We kept the families in a greenhouse at the University of Missouri (Columbia, MO, USA) and caged each family separately on a potted *Albizia julibrissin* (Mimosaceae) host plant. Approximately 1 wk after a family eclosed to adulthood, we caged the sexes on different *A. julibrissin* plants to prevent sibling mating. We produced the families used in this study through selective mating of unrelated individuals.

Study Design

We measured male mate-searching behaviours once per week over 4 consecutive weeks beginning at the onset of male sexual maturity, i.e. at 12, 19, 25, and 33 d old post-adult eclosion. We conducted the experiment between Nov. 2006 and Jan. 2007, in a temperature controlled room ($25 \pm 2°C$) at a greenhouse at the University of Missouri. We kept humidity between 40% and 50% using a humidifier and set the photoperiod for a 13:11 (L:D) cycle.

We used two cages ($145 \times 68 \times 92$ cm) that we monitored simultaneously. Within each cage, we placed two potted *A. julibrissin* plants (approximate plant height = 80 cm) at opposite ends. We marked each *A. julibrissin* plant with coloured nail polish in 5 cm intervals along its stem, branches and petioles to measure within-plant walking rates (see 'Mate-searching behaviour' below). This method of marking the substrate has been used in previous studies with treehoppers and does not affect either plant

health or treehopper behaviour (Sullivan-Beckers & Cocroft 2010).We used the same plants in each cage for the entire duration of the experiment. After each set of observations was completed, we washed the plants with a mild detergent soap to remove any potential chemical cues left by the previous group of insects, and we re-marked the plants with nail polish as needed.

We examined males from 12 different families. We randomly selected individuals from their respective family groups for use in this study. We first placed six adult sibling females into each cage (three per plant), placing them near the tops of the plants. Females did not move off the plant they were placed on, and tended to remain in the same location near the top of the plant where the foliage was thickest throughout an observation period. Females were 10–15 d old post-eclosion and so were not yet sexually mature (see 'Courtship behaviour' below). We kept female age constant over the duration of the experiment by using different females each week of the correct age drawn from our greenhouse colony. Once females were in the cages, we then immediately placed six sibling males (three per plant), near the base of the plants. Males were not related to females within the same cage. We marked each male with a numbered plastic tag (3 mm²) glued to the left side of the pronotum. We allowed insects to settle for approximately 1 h before observations began. When occasional mortality reduced the number of males in a family, we reduced the number of females in that cage accordingly to maintain an equal sex ratio within the cages. This ratio occurs in natural populations in Florida where our colony was derived (Wood & Dowell 1984).

When focal males were not being observed, we kept them with their brothers on a potted *A. julibrissin* plant enclosed within a screen cage in another room in the greenhouse. During this time, they were kept with an equal number of unrelated females. We did this to provide males the opportunity to interact with females as they would in nature, so that during the observation periods the behaviours we observed would be as natural as possible, and not the result of sexually mature (and naïve) males being isolated from females for extended periods of time.

Male Condition

We weighed males individually on an electronic balance (Ohaus AS64) to the nearest 0.1 mg prior to placing them into a cage for observations. Because it was necessary to glue the identification tag to the males prior to beginning observations, we subtracted the tag's weight from the male's weight before calculating its condition. We calculated condition for each male each week by taking the ratio of body mass to an index of volume (Cotton et al. 2004).The pronotum of *U. crassicornis* (as with all species of treehoppers) extends over the entire dorsal surface of the body and thus provides a good approximation of body volume (Wood & Morris 1974). Accordingly, we multiplied three measures of the pronotum (length, width, and height) to estimate body volume. We measured each male's pronotum twice to the nearest ±0.01 mm with Manostat calipers, and we used the average values in the analysis.

Mate-Searching Behaviour

We examined age-based changes in flight activity and within-plant movement. For each of the four age classes, we made observations of both cages every 15 min for 3 consecutive hours. During observations, we recorded the location of each male as well as the occurrence of any courtship encounter. Recording male locations in each cage usually required approximately 5 min; we spent the remaining time conducting scan sampling of males in both cages to monitor the occurrence of courtship behaviour.

We assessed flight activity for each male by observing how many times a male flew off the plants during an observation period. We divided the number of flights by the total amount of time spent on the plants (No. flights/min) to obtain a flight rate. Under natural conditions, males will fly from one plant to another. In the cages, flight paths were sometimes interrupted when males encountered the screen. If a male flew from a plant to the screen and then flew back to the same plant, we omitted those two flight events from our counts. However, if a male flew to the screen and then flew to other plant, we included that sequence as one flight event in our calculations. We averaged the data for all males within the cage to obtain a family mean.

We assessed within-plant movement by calculating how far each male moved over the 3 h period. We measured the total distance a male walked by using the marks of nail polish on the stem, branches, and petioles to record a male's precise location at each of the 15-min sampling periods, and then we summed his total movement on the plant starting from his initial location at the plant's base. Preliminary observations of male movement rates suggested

that sampling male locations as described above provided a reliable index of within-plant movement. Occasionally, some males would walk from a plant to the side or top of the screen cage, but these off-plant movements were excluded to eliminate any potential influence of the artificial substrate on a male's walking behaviour. This reduced total (plant + screen) walking movement by 51% [3.98 ± 2.09 cm per male ($\bar{x}$ ± SD); n = 72 males], but it did not alter the results with respect to age-related movement patterns. For each male, we calculated his movement rate (cm moved/min) and then averaged the data for all males within the cage to obtain a family mean.

Courtship Behaviour

Male *U. crassicornis* will court both receptive and unreceptive females (Wood 1974; De Luca & Cocroft 2008). Furthermore, in their natural habitat males are more likely to encounter and court unreceptive females during mate searching (Wood & Dowell 1985). We therefore decided to use immature, unreceptive females which are unresponsive to male courtship. Receptive females vary in the length of time they permit a male to court before actively accepting or rejecting him, and mated females will immediately reject a courting male by shaking him off (PA De Luca, own data). Our goal was to isolate the effect of age on the duration of male courtship, so by using sexually unreceptive females any variation among males would not be attributed to variation in female behaviour as a result of differences in receptivity. For each male that courted, we first measured the time from the start of the observation period to when he was observed courting for the first time. This provided a measure of how quickly males were able to find a female and initiate courtship. We then measured the duration of each courtship encounter, starting from the time the male climbed onto the female to when he dismounted. We averaged courtship durations for all males within a cage to obtain a family mean.

Statistical Analyses

Our experimental unit for all statistical analyses was the mean response of males from one family (n = 12). Parametric statistical analyses were conducted using JMP IN v.5.1 (SAS Institute Inc. 2003). We assessed age-based changes in male condition using a repeated-measures ANOVA with age (fixed effect) and family identity (random effect) as factors.

We log-transformed condition to achieve normality (Shapiro–Wilk W = 0.993, p = 0.35) and homogeneity of variance (Bartlett test, p = 0.83).

We examined the effect of age on flight activity using a non-parametric Quade test as the data could not be normalized with an appropriate transformation (Shapiro–Wilk W = 0.539, p < 0.0001; Bartlett test, p < 0.0001). This test is analogous to the Wilcoxon signed rank test for more than two groups and also allows for post-hoc comparisons between treatment levels using an analysis specific to the Quade test (Conover 1980).

We examined the effect of age on within-plant movement using a repeated-measures ANOVA with age (fixed effect) and family identity (random effect) as factors. We log-transformed within-plant movement rate to achieve normality (Shapiro–Wilk W = 0.981, p = 0.62), and homogeneity of variance (Bartlett test, p = 0.68).

We evaluated age-based differences in the time it took males to locate a female for courtship. However, males in some families courted at only one age, and males in other families never courted; thus, the data were unbalanced both within and between age classes. Accordingly, we used a non-parametric Durbin test. This test is similar to the Quade test, but is appropriate when there are missing cells in the data (Conover 1980). We also used a Durbin test to examine the effect of age on courtship duration, as there were missing cells in that data set. We did not include data from males that did not court (i.e. courtship duration = 0 min) in our analyses. In some cases, 33-d-old males continued to court even when the 3 h observation period ended. When this occurred, we did not disturb the individuals to allow the courtship sequence to proceed to its own conclusion.

Results

Condition was highest for 12-d-old males and declined as males reached 19 d old but then stabilized ($F_{3,33}$ = 13.97, p < 0.0001, Fig. 1A). Pair-wise multiple comparisons revealed differences only between males aged 12 and 19, 12 and 25, and 12 and 33 d (α = 0.05, critical t = 2.03 for multiple comparisons: 12 vs. 19 d, t = 4.49; 12 vs. 25 d, t = 5.43; 12 vs. 33 d, t = 5.64; all p < 0.0001). Mortality reduced the number of males over the course of the experiment. The mean proportion of surviving males at 19, 25, and 33 d was 0.72, 0.66, and 0.61, respectively. No family suffered complete mortality before the end of the study period.

Flight rates were highest for 12- and 19-d-old males and then decreased for 25 and 33 d old males (Quade test: n = 12 families, t = 4.62, p < 0.05; Fig. 1B). Males never flew between locations on the same plant, but rather flew between plants or from a plant to the cage screen. Pair-wise multiple comparisons revealed differences only between males aged 12 and 25, 12 and 33, 19 and 25, and 19 and 33 d (α = 0.05, critical t = 82.01 for multiple comparisons: 12 vs. 25 d, t = 116.5; 12 vs. 33 d, t = 97.5; 19 vs. 25 d, t = 114.5; 19 vs. 33 d, t = 99.5; all p < 0.05).

Within-plant movement varied with age ($F_{3,33}$ = 3.83, p = 0.02, Fig. 1C). Walking rates increased as males aged from 12 to 19 d and then stabilized. Pair-wise multiple comparisons revealed differences only between males aged 12 and 19, and 12 and 25 d (α = 0.05, critical t = 2.03 for multiple comparisons: 12 vs. 19 d, t = 2.98; 12 vs. 25 d, t = 3.07; all p < 0.005).

We never observed males from the youngest age class (12 d) engaging in courtship behaviour. We recorded courtship events from males from eight families only. The time it took a male to find a female and initiate courtship increased significantly from 19 to 25 d and then stabilized (Durbin test: n = 8 families, t = 6.5, p = 0.039, Fig. 1D). Pair-wise multiple comparisons revealed significant differences only between males aged 19 and 25, and 19 and 33 d (α = 0.05, critical t = 1.89 for multiple comparisons: 19 vs. 25 d, t = 4, p < 0.05; 19 vs. 33 d, t = 6, p < 0.05).

We observed a total of 88 courtships. Within each age class, there were 33 (19 d old, n = 52 males), 41 (25 d old, n = 48 males), and 14 (33 d old, n = 44 males) courtships observed. There was no significant difference in average courtship duration between age classes (Durbin test: n = 8 families, t = 4, p = 0.13, Fig. 1E), although 33-d-old males had the longest maximum courtship times,
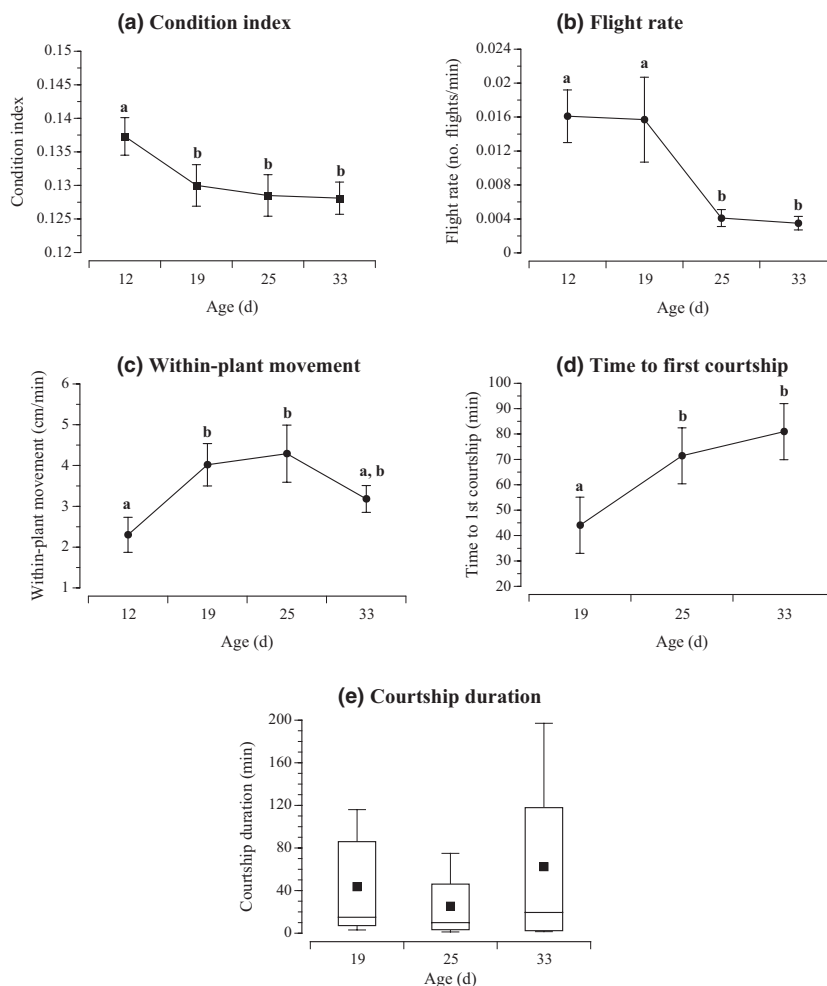

Fig. 1: The effect of age on different aspects of mate acquisition behaviour in *Umbonia crassicornis*. (A) Physical condition. (B) Flight rate. (C) Within-plant movement. (D) Time to first courtship. (E) Courtship duration. Graphs A–D show the $\bar{x} \pm$ SE. Box-plot in E shows the 90th, 75th, median, 25th, and 10th percentiles, with black squares representing the mean. For all graphs, letters indicate age groups that were significantly different following post-hoc tests.

which in three cases exceeded the 3 h observation period.

Discussion

In thornbug treehoppers, age has a significant effect on mate-searching behaviour. In contrast to our first prediction, older (25 and 33 d) males did not increase mate-searching effort relative to younger (19 d) males even though their condition did not decrease. Rather, younger males found and initiated courtship with females in approximately half the time required by older males, possibly as a result of their greater rates of plant-to-plant flight. Our discussions below are limited to comparisons among the three oldest age classes (19, 25, and 33 d old) as it is within this age range that the complete repertoire of mate acquisition behaviour in *U. crassicornis* was expressed. The high level of flight activity and low within-plant movement rates for 12-d-old males are consistent with the onset of dispersal that occurs at this age (Wood & Dowell 1985; Masters 1997). Furthermore, the complete lack of courtship behaviour indicated that 12-d-old males were not yet searching for females.

Although condition remained stable for males aged 19–33 d, the sharp decline in flight activity after 25 d suggests that some (as yet undetermined) factor affected the ability of older males to sustain high flight rates. The ratio of mass to volume we used to characterize condition is one of several indices that are used (Otronen 1995; Blanckenhorn & Hosken 2002; Kotiaho 2002; Cotton et al. 2004), yet this condition proxy does not explain the decline in flight activity for older thornbug males. It is therefore possible that other age-related physiological processes are affecting older males so their flight activity is reduced. For example, changes in the structure and composition of insect flight muscles are commonly linked to a decrease in flight in older males (Sohal 1976; Collatz & Wilps 1986; Casey 1989; Matsubara et al. 2005). Reductions in carbohydrate and lipid reserves used to supply flight muscles with energy may also affect flight activity (Kutsch 1989; Marden & Rollins 1994; Otronen 1995; Marden 2000; Roberts & Elekonich 2005), although changes in mass from 19 to 33 d were not large enough to indicate that older males had lost significant energy reserves. Whatever the cause of reduced flight activity, within-plant walking rates did not undergo the same age-related decline, which suggests that walking behaviour may not be as sensitive to the kinds of physiological processes that reduce flight activity.

Nevertheless, as male thornbugs must actively seek out stationary females, the walking rates exhibited by older males were not nearly sufficient to compensate for their low flight activity as evidenced by the longer search times needed to locate females.

What might explain the higher female encounter rates of younger males, given that they did not have higher rates of within-plant movement? Our sampling method did not provide us with individual search paths, but we hypothesize that the success of younger males in locating females is because of their greater rates of plant-to-plant flight. This could be the case if flying males are guided (perhaps by visual cues) to sites where females are most likely to be encountered. Female thornbugs are typically found near the growing tips of branches in their natural habitat (Wood & Dowell 1985; Masters 1997; Cocroft 2002), so any cues that would allow the insects to identify new growth (see Coley & Barone 1996) would also bias searches towards likely female locations (see Hunt & Nault 1991). Flight may increase encounter rates relative to within-plant walking, which might take longer as a consequence of plant architecture (e.g. a male moving from one branch tip to the next must traverse the length of both branches and the intervening main stem). Although older males would also be expected to fly towards probable female locations, their flight rates were over four times lower than those of younger males. Given that female thornbugs mate only once in their lifetime, younger males that are able to locate a dispersed female first are thus likely to have a significant mating advantage over older rivals.

The lack of a difference in average courtship durations between age classes was unexpected, especially because condition did not decline as males aged. In many species older males often display a longer willingness to persist in reproductive behaviours such as courtship, even when they are in poor condition (Woodhead 1986; Andersson 1994; Felton et al. 2006; Kemp et al. 2006; Fischer et al. 2008; Kemp 2008; Engqvist 2009). Although some males in the oldest age class courted for the longest maximum times (e.g. 200 min vs. 120 min for 19 d old males), the low number of courtships suggests that older males were not increasing courtship effort in response to low flight activity. Furthermore, given that courtship durations varied greatly within age groups, it appears that males were making choices regarding courtship investment that were not age-dependent. Instead, the time a male devoted to courtship might have been based on an assessment of the cost/benefit ratio of actively searching for

receptive females vs. stationary courtship of a female of unknown receptivity status (Parker 1974, 1978; Engqvist 2009). Some males (regardless of age) may therefore have been more willing to remain with a female for several hours and devote their effort to courtship, rather than continued mate searching, whereas other males chose the opposite approach. If males were aware that females were unreceptive courtship times should have been uniformly short, but average times ranged from 30 to 60 min across age classes, suggesting that males were unable to determine a female's receptivity status. It is possible that low courtship times were the result of males becoming familiar with females in the cage, and thus promptly terminating an encounter with a previously courted female (Jordan & Brooks 2010). Another factor that may have contributed to variation in courtship duration among males was interference competition. Females in this study were sometimes courted by two males simultaneously, a situation that is common in natural populations (Wood 1974; Wood & Dowell 1985). Although we did not assess the extent of male competition during courtship, it is possible that for some males courtship decisions were influenced by the presence of other competing males in the vicinity (Schafer & Uhl 2003; Radwan et al. 2005; Wong & Candolin 2005), although additional research is needed to test this hypothesis.

One aspect of thornbug searching behaviour that we did not evaluate for age-related increases in effort was mate advertisement signalling. Establishing a duet with a sexually receptive female is one way for a male to achieve pair formation in *U. crassicornis* (Cocroft & McNett 2006; De Luca & Cocroft 2009). However, in their natural habitat males must also conduct mate searching without the benefit of female reply vibrations (Wood & Dowell 1985), and our experiment was specifically designed to investigate age effects on searching behaviour under these conditions. Accordingly, the relationship between age and signalling effort remains an avenue for future research in this species. Age-related increases in signalling effort occur in many species (reviewed in Brooks & Kemp 2001), and if older thornbug males increased calling rates when searching within a plant, it could compensate for their reduced flight activity if it helped to increase encounter rates with sexually responsive females (Scheffer et al. 1996; Bailey 2003; Miranda 2006; Stewart & Sandberg 2006).

In conclusion, our study reveals that flight activity is more important than within-plant walking for mate-searching thornbug males when females are sparsely distributed, and furthermore, males may be investing so heavily in flight at younger ages that they are unable to allocate resources to maintain high flight activity when they get older. Accordingly, these findings imply a mating advantage for younger thornbug males when active searching is required to locate dispersed females. However, a previous study with *U. crassicornis* found that older males are more successful at mating when females are tightly aggregated in family groups (De Luca & Cocroft 2008). We offer an explanation to reconcile the present results with those of the former study. Recall that females can mate in one of two social contexts: while still in their natal aggregation or after dispersing (Wood 1974; Wood & Dowell 1985). For pre-dispersal females that are still aggregated with their family, mating decisions are biased towards older males, probably because older males are more frequent visitors to these high-density aggregations and perform more courtships here as well (Wood & Dowell 1985; De Luca & Cocroft 2008). However, under low-density conditions, i.e. when females are dispersed, mating likely favours younger males as their greater rates of plant-to-plant flight enable them to locate females much faster. Accordingly, whether younger or older thornbug males are more successful at mating may depend greatly on the social environment (high vs. low female density) experienced during mate searching, in addition to individual differences among males in their ability to solicit matings from females.

Acknowledgements

Literature Cited

Alonso, J. C., Magana, M., Palacin, C. & Martin, C. A. 2010: Correlates of mating mating success in great bustard leks: the effects of age, weight, and display effort. Behav. Ecol. Sociobiol. **64**, 1589—1600.

Andersson, M. 1994: Sexual Selection. Princeton Univ. Press, New Jersey.

Bailey, W. J. 2003: Insect duets: underlying mechanisms and their evolution. Physiol. Entomol. **28**, 157—174.

Bertram, S. M. 2000: The influence of age and size on temporal mate signalling behaviour. Anim. Behav. **60**, 333—339.

Bitton, P. P., O'Brien, E. L. & Dawson, R. D. 2008: Plumage brightness and age predict extrapair fertilization success of male tree swallows, *Tachycineta bicolor*. Anim. Behav. **74**, 1777—1784.

Blanckenhorn, W. U. & Hosken, D. J. 2002: Heritability of three condition surrogates in the yellow dung fly. Behav. Ecol. **14**, 612—618.

Bonsall, M. B. & Mangel, M. 2004: Life-history trade-offs and ecological dynamics in the evolution of longevity. Proc. R. Soc. Lond. B Biol. Sci. **271**, 1143—1150.

Brooks, R. & Kemp, D. J. 2001: Can older males deliver the good genes? Trends Ecol. Evol. **16**, 308—313.

Brown, G. P. & Weatherhead, P. J. 1999: Female distribution affects mate searching and sexual selection in male northern water snakes (*Nerodia sipedon*). Behav. Ecol. Sociobiol. **47**, 9—16.

Casey, T. M. 1989: Development of the flight motor pattern. In: Insect Flight (Goldsworthy, G. J. & Wheeler, C. H., eds). CRC Press, Boca Raton, pp. 257—272.

Claridge, M. F. 1985: Acoustic signals in the homoptera: behavior, taxonomy, and evolution. Annu. Rev. Entomol. **30**, 297—317.

Claridge, M. F. & de Vrijer, P. W. F. 1994: Reproductive behavior: the role of acoustic signals in species recognition and speciation. In: Planthoppers: Their Ecology and Management (Denno, R. F. & Perfect, T. J., eds). Chapman and Hall, Inc., New York, pp. 216—233.

Cocroft, R. B. 2002: Antipredator defense as a limited resource: unequal predation risk in broods of an insect with maternal care. Behav. Ecol. **13**, 125—133.

Cocroft, R. B. 2003: The social environment of an aggregating, ant-attended treehopper (Hemiptera: Membracidae: *Vanduzea arquata*). J. Insect Behav. **16**, 79—95.

Cocroft, R. B. & McNett, G. D. 2006: Vibrational communication in treehoppers (Hemiptera: Membracidae). In: Insect Sounds and Communication: Physiology, Behaviour, Ecology and Evolution (Drosopolous, S. & Claridge, M. F., eds). CRC Press, Boca Raton, pp. 321—333.

Cökl, A., Virant-Doberlet, M. & McDowell, A. 1999: Vibrational directionality in the southern green stink bug, *Nezara viridula* (L.), is mediated by female song. Anim. Behav. **58**, 1277—1283.

Coley, P. D. & Barone, J. A. 1996: Herbivory and plant defenses in tropical forests. Annu. Rev. Ecol. Syst. **27**, 305—335.

Collatz, K. G. & Wilps, H. 1986: Aging of the flight mechanism. In: Insect Aging (Collatz, K. G. & Sohal, R. S., eds). Springer-Verlag, Berlin, pp. 55—72.

Conner, J. 1989: Older males have higher insemination success in a beetle. Anim. Behav. **38**, 503—509.

Conover, W. J. 1980: Practical Non-Parametric Statistics. John Wiley & Sons, Inc., New York.

Cotton, S., Fowler, K. & Pomiankowski, A. 2004: Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? Proc. R. Soc. Lond. B Biol. Sci. **271**, 771—783.

De Fraipont, M., Fitzgerald, G. J. & Guderley, H. 1993: Age-related differences in reproductive tactics in the three-spined stickleback, *Gasterosteus aculeatus*. Anim. Behav. **46**, 961—968.

De Luca, P. A. & Cocroft, R. B. 2008: The effects of age and relatedness on mating patterns in thornbug treehoppers: inbreeding avoidance or inbreeding tolerance? Behav. Ecol. Sociobiol. **62**, 1869—1875.

De Luca, P. A. & Cocroft, R. B. 2009: Age-related changes in an insect mating signal have no effect on female choice. Behav. Ecol. Sociobiol. **63**, 1787—1798.

Demary, K., Michaelidis, C. I. & Lewis, S. M. 2006: Firefly courtship: behavioral and morphological predictors of male mating success in *Photinus greeni*. Ethology **112**, 485—492.

DeWinter, A. J. & Rollenhagen, T. 1990: The importance of male and female acoustic behaviour for reproductive isolation in *Ribautodelphax* planthoppers (Homoptera: Delphacidae). Biol. J. Linn. Soc. **40**, 191—206.

Diaz, E. R. & Thiel, M. 2004: Chemical and visual communication during mate searching in rock shrimp. Biol. Bull. Mar. Biol. Lab. Woods Hole **206**, 134—143.

Dunn, P. O. & Whittingham, L. A. 2007: Search costs influence the spatial distribution, but not the level, of extra-pair mating in tree swallows. Behav. Ecol. Sociobiol. **61**, 449—454.

Duvall, D. & Schuett, G. W. 1997: Straight-line movement and competitive mate searching in prairie rattlesnakes, *Crotalus viridis viridis*. Anim. Behav. **54**, 329—334.

Engqvist, L. 2009: Should I stay or should I go? Condition- and status-dependent courtship decisions in the scorpionfly Panorpa cognata. Anim. Behav. **78**, 491—497.

Felton, A., Alford, R. A., Fenton, A. M. & Schwarzkopf, L. 2006: Multiple mate choice criteria and the importance of age for male mating success in the microhylid

frog, *Cophixalus ornatus*. Behav. Ecol. Sociobiol. **59**, 786—795.

Fischer, J., Kitchen, D. M., Seyfarth, R. M. & Cheney, D. L. 2004: Baboon loud calls advertise male quality: acoustic features and their relation to rank, age, and exhaustion. Behav. Ecol. Sociobiol. **56**, 140—148.

Fischer, K., Perlick, J. & Galetz, T. 2008: Residual reproductive success and male mating success: older males do better. Proc. R. Soc. Lond. B Biol. Sci. **275**, 1517—1524.

Forbes, M. R., Boates, J. S., McNeil, N. L. & Brison, A. E. 1996: Mate searching by males of the intertidal amphipod *Corophium volutator* (Pallas). Can. J. Zool. **74**, 1479—1484.

Forstmeier, W., Hasselquist, D., Bensch, S. & Leisler, B. 2005: Does song reflect age and viability? A comparison between two populations of the great reed warbler Acrocephalus arundinaceus. Behav. Ecol. Sociobiol. **59**, 634—643.

Garamszegi, L. Z., Torok, J., Hegy, G., Szollosi, E., Rosivall, B. & Eens, M. 2007: Age-dependent expression of song in the Collared Flycatcher, *Ficedula albicollis*. Ethology **113**, 246—256.

Gil, D., Cobb, J. & Slater, P. J. B. 2001: Song characteristics are age dependent in the willow warbler, *Phylloscopus trochilus*. Anim. Behav. **62**, 689—694.

Hedrick, A. V. 1986: Female preferences for male calling bout duration in a field cricket. Behav. Ecol. Sociobiol. **19**, 73—77.

Höglund, J. & Lundberg, A. 1987: Sexual selection in a monomorphic lek-breeding bird: correlates of male mating success in the great snipe *Gallinago media*. Behav. Ecol. Sociobiol. **21**, 211—216.

Howard, R. D. 1978: The evolution of mating strategies in bullfrogs, *Rana catesbeiana*. Evolution **32**, 850—871.

Hunt, R. E. 1994: Vibrational signals associated with mating behavior in the treehopper, *Enchenopa binotata* Say (Hemiptera: Homoptera: Membracidae). J. NY Entomol. Soc. **102**, 266—270.

Hunt, R. E. & Nault, L. R. 1991: Roles of interplant movement, acoustic communication, and phonotaxis in mate-location behavior of the leafhopper *Graminella nigrifrons*. Behav. Ecol. Sociobiol. **28**, 315—320.

Hunt, J., Brooks, D. R., Jennions, M. D., Smith, M. J., Bentsen, C. L. & Bussière, L. F. 2004: High-quality male field crickets invest heavily in sexual display but die young. Nature **432**, 1024—1027.

Jacot, A., Scheuber, H. & Brinkhof, M. 2007: The effect of age on a sexually selected acoustic display. Ethology **113**, 615—620.

Jordan, L. A. & Brooks, R. C. 2010: The lifetime costs of increased male reproductive effort: courtship, copulation and the Coolidge effect. J. Evol. Biol. **23**, 2403—2409.

Judge, K. A., Ting, J. L. & Gwynne, D. T. 2008: Condition dependence of male life span and calling effort in a field cricket. Evolution **62**, 868—878.

Kasumovic, M. M., Bruce, M. J., Herberstein, M. E. & Andrade, M. C. B. 2007: Risky mate search and mate preference in the golden orb-web spider (*Nephila plumipes*). Behav. Ecol. **18**, 189—195.

Kemp, D. J. 2008: Female mating biases for bright ultraviolet iridescence in the butterfly *Eurema hecabe* (Pieridae). Behav. Ecol. **19**, 1—8.

Kemp, D. J., Wiklund, C. & Gotthard, K. 2006: Life history effects upon contest behaviour: age as a predictor of territorial contest dynamics in two populations of the Speckled Wood Butterfly, *Pararge aegeria* L. Ethology **112**, 471—477.

Kokko, H. 1997: Evolutionarily stable strategies of age-dependent sexual advertisement. Behav. Ecol. Sociobiol. **41**, 99—107.

Kotiaho, J. 2002: Sexual selection and condition dependence of courtship display in three species of horned dung beetles. Behav. Ecol. **13**, 791—799.

Kutsch, W. 1989: Development of the flight motor pattern. In: Insect Flight (Goldsworthy, G. J. & Wheeler, C. H., eds). CRC Press, Boca Raton, pp. 51—73.

Marden, J. H. 2000: Variability in the size, composition, and function of insect flight muscles. Annu. Rev. Physiol. **62**, 157—178.

Marden, J. H. & Rollins, R. A. 1994: Assessment of energy reserves by damselflies engaged in aerial contests for mating territories. Anim. Behav. **48**, 1023—1030.

Masters, K. L. 1997: Behavioral and Ecological Aspects of Inbreeding in Natural Animal Populations: Inferences from Umbonia Treehoppers (Homoptera: Membracidae). Ph.D. Dissertation, Princeton Univ., Princeton.

Matsubara, K., Tojo, S. & Suzuki, N. 2005: Age-related changes in flight muscle mass, lipid reserves and flight capability during adult maturation in males of the territorial damselfly *Calopteryx atrata* (Odonata: Calopterygidae). Zool. Sci. **22**, 587—592.

McKamey, S. H. & Deitz, L. L. 1996: Generic revision of the New World tribe Hoplophorionini (Hemiptera: Membracidae: Mebracinae). Syst. Entomol. **21**, 295—342.

Mead, F. W. 1962: The thorn bug, *Umbonia crassicornis* (Amyot and Serville) (Homoptera: Membracidae). Fla. Dep. Agric. Consum. Serv. Div. Plant Ind. Entomol. Cir. No. 8.

Miller, L. K. & Brooks, R. 2005: The effects of genotype, age, and social environment on male ornamentation, mating behavior, and attractiveness. Evolution **59**, 2414—2425.

Miranda, X. 2006: Substrate-borne signal repertoire and courtship jamming by adults of *Ennya chrysura*

(Hemiptera: Membracidae). Ann. Entomol. Soc. Am. **99**, 374—386.

Otronen, M. 1995: Energy reserves and mating success in males of the yellow dung fly, *Scathophaga stercoraria*. Funct. Ecol. **9**, 683—688.

Parker, G. A. 1974: Courtship persistence and female guarding as male time investment strategies. Behaviour **48**, 157—187.

Parker, G. A. 1978: Searching for mates. In: Behavioural Ecology: An Evolutionary Approach (Krebs, J. R. & Davies, N. B., eds). Blackwell Scientific Publications, Oxford, pp. 214—244.

Radwan, J., Michalczyk, L. & Prokop, Z. 2005: Age dependence of male mating ability and sperm competition success in the bulb mite. Anim. Behav. **69**, 1101—1105.

Ritchie, M. G., Couzin, I. D. & Snedden, W. A. 1995: What's in a song? Female bushcrickets discriminate against the song of older males. Proc. R. Soc. Lond. B Biol. Sci. **262**, 21—27.

Roberts, S. P. & Elekonich, M. M. 2005: Muscle biochemistry and the ontogeny of flight capacity during behavioral development in the honey bee, *Apis mellifera*. J. Exp. Biol. **208**, 4193—4198.

SAS Institute Inc. 2003: JMP IN: The Statistical Discovery Software: Release 5.1. SAS Institute, Inc., Cary, NC.

Schafer, D. & Uhl, G. 2003: Male competition over access to females in a spider with last-male sperm precedence. Ethology **109**, 385—400.

Scheffer, S. J., Uetz, G. W. & Stratton, G. E. 1996: Sexual selection, male morphology, and the efficacy of courtship signalling in two wolf spiders (Araneae:Lycosidae). Behav. Ecol. Sociobiol. **38**, 17—23.

Shuster, S. M. & Wade, M. J. 2003: Mating Systems and Strategies. Princeton Univ. Press, Princeton.

Sikkel, P. C. 1998: Competitor intrusions and mate-search tactics in a territorial marine fish. Behav. Ecol. **9**, 439—444.

Sohal, R. S. 1976: Aging changes in insect flight muscle. Gerontology **22**, 317—333.

Stearns, S. C. 1992: The Evolution of Life Histories. Oxford Univ. Press, New York.

Stewart, K. W. & Sandberg, J. B. 2006: Vibratory communication and mate searching behaviour in stoneflies. In: Insect Sounds and Communication: Physiology, Behaviour, Ecology and Evolution (Drosopoulos, S. & Claridge, M., eds). CRC, Boca Raton, pp. 179—186.

Sullivan-Beckers, L. E. & Cocroft, R. B. 2010: The importance of female choice, male-male competition, and signal transmission as causes of selection on male mating signals. Evolution **64**, 3158—3171.

Uy, J. A. C., Patricelli, G. L. & Borgia, G. 2001: Complex mate searching in the satin bowerbird *Ptilonorhynchus violacius*. Am. Nat. **158**, 530—542.

Wong, B. & Candolin, U. 2005: How is female mate choice affected by male competition? Biol. Rev. **80**, 559—571.

Wood, T. K. 1974: Aggregating behavior of *Umbonia crassicornis* (Homoptera: Membracidae). Can. Entomol. **106**, 169—173.

Wood, T. K. & Dowell, R. 1984: Sex ratio in *Umbonia crassicornis* Amyot and Serville (Homoptera: Membracidae). Am. Midl. Nat. **112**, 58—66.

Wood, T. K. & Dowell, R. 1985: Reproductive behavior and dispersal in *Umbonia crassicornis* (Homoptera: Membracidae). Fla. Entomol. **68**, 151—158.

Wood, T. K. & Morris, G. K. 1974: Studies on the function of the Membracid pronotum (Homoptera) I. Occurrence and distribution of articulated hairs. Can. Entomol. **106**, 143—148.

Woodhead, A. P. 1986: Male age: effect on mating behaviour and success in the cockroach *Diploptera punctatai*. Anim. Behav. **34**, 1874—1879.

Woolbright, L. L., Greene, E. J. & Rapp, G. C. 1990: Density-dependent mate searching strategies of male wood frogs. Anim. Behav. **40**, 135—142.

Zeigler, D. D. & Stewart, K. W. 1985: Age effects on drumming behavior of *Pteronarcella badia* (Plecoptera) males. Entomol. News **96**, 157—160.