



# Effects of Age and Experience on Male Mate Choosiness

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## Abstract

Mate choosiness by males has been documented in many taxa but we still do not know how it varies with age even though such variation can be important for our understanding of sexual selection on females. Theory provides conflicting predictions: young males, who are less attractive to females than older males, may be less choosy, or older males, who face fewer expected future mating opportunities, may be less choosy. In our experiments with fruit flies (*Drosophila melanogaster*), young (1-d-old) males spent relatively less time courting recently mated females than did mature (4-d-old) males. Overall, there was a gradual decline in male mate choosiness from age 1–7 d. As male age was correlated with the duration of deprivation from females, we tested for the effect of deprivation and found that same-age males previously exposed to females were choosier than female-deprived males. We also assessed key male parameters that could affect choosiness and found that, compared to mature males, young males were less attractive to females, less competitive in intramale interactions and less fertile. Although the lesser attractiveness and competitiveness should select for lesser mate choosiness in young males, their limited fertility and more expected future mating opportunities seem to override the other factors and lead to high mate choosiness in young males. Overall, our data indicate that young males just after reaching sexual maturity are choosy and that subsequent exposure to females can maintain high levels of male mate choosiness with age. Hence, males can contribute much more to sexual selection than previously appreciated.

## Introduction

It is now well established that male mate choice is prevalent even in species where males provide only sperm (Bonduriansky 2001; Edward & Chapman 2011). Examples include water isopods (*Asellus* spp.) (Manning 1975), Fruit flies (*Drosophila melanogaster*) (Cook & Cook 1975), fowl (*Gallus gallus*) (Pizzari et al. 2003), mice (*Mus musculus domesticus*) (Ramm & Stockley 2014) and monkeys (*Macaca mulatta*) (Herbert 1968). Male mate choice in such species indicates that, even in such taxa, males are sometimes limited by factors other than access to females. Such factors may include time, sperm or seminal fluid, and the mortality costs of courtship and mating (Dewsbury 1982; Schwagmeyer & Parker 1990; Van Voorhies 1992; Wedell et al. 2002). Indeed, experiments in a

variety of species indicate that courtship is associated with increased mortality rate (Cordts & Partridge 1996; Clutton-Brock & Langley 1997; Kotiaho 2000), that sperm-limited males are more choosy than males with large sperm stores (Byrne & Rice 2006; Long et al. 2009) and that males strategically allocate more sperm to mated, large, attractive and young females than to virgin, small, unattractive or old females, respectively (Wedell et al. 2002; Pizzari et al. 2003; Lüpold et al. 2011; Ramm & Stockley 2014).

The realization that even males in settings with a highly male-biased operational sex ratio can benefit from exercising choice owing to the costs of courtship and mating paves the way to analyses of the optimal allocation of males' resources to courting and mating based on the expected increments in fitness that mating with a given female can provide (Kokko &

Monaghan 2001; Kokko et al. 2006). Indeed, research on male mate choice indicates that males often prefer females that are more fertile as indicated by their body size, age and current mating status. Examples include male preference for larger, younger and virgin females in a wide variety of taxa (Bonduriansky 2001; Wedell et al. 2002; Edward & Chapman 2011). Another factor that should affect male mate choice is the likelihood of succeeding in mating with a given female, especially in taxa where females accept males only after an extensive period of courtship.

In addition to the female characteristics that influence male mate choice, males' optimal investment in mating effort and their level of mate choosiness may vary with their own traits, including relative quality, social status and age. While there has been significant research effort devoted to assessing age-specific allocation of resources to reproduction (Clutton-Brock 1988; Roff 1992; Stearns 1992), much of this work has focused on females. We are not aware of life history models addressing specifically the optimal allocation of resources to reproduction with age in males, and it is not obvious to what degree the theory focusing on females is relevant for males. The limited experimental data on mate choice as a function of male age are inconclusive. Martel et al. (2008) reported no effect of age on choosiness in males of the parasitoid wasp *Trichogramma turkestanica* presented with virgin and inseminated females. Two related studies in fruit flies (*D. melanogaster*) documented, first, that there were slightly higher pairings (54% vs. 46%) between males and the more fertile out of two available females (Edward & Chapman 2012) and that there was little variation with male age in the potential benefit from mate choice (Edward & Chapman 2013). Finally, in our own work, we found that young, 1-d-old male fruit flies (*D. melanogaster*) showed higher levels of mate choosiness than mature, 4-d-old males. Specifically, while the young males spent as much time as the mature males pursuing conspecific females, they devoted significantly less time to courting females of the closely related species *D. simulans*. We replicated these findings with two distinct lines of *D. melanogaster*, using a variety of protocols including no-choice and choice set-ups, and testing inexperienced and experienced males. We suggested that the mature males were less choosy than the young males because they had experienced a longer period of female deprivation prior to the tests (Dukas & Baxter 2014).

Our data indicating variation in male mate choosiness with age called for a few follow-up experiments. First, the fact that young males were rather selective

and mostly avoided courting heterospecific females is highly relevant for research on incipient speciation (Peterson et al. 2005; Servedio & Dukas 2013). To assess the pertinence of our findings for work on sexual selection on females, we wished to quantify male mate choosiness when encountering distinct categories of conspecific females. To this end, we tested how male age influences mate choosiness when encountering either small vs. large females or virgin vs. recently mated females. Second, to refine our analysis of male age and mate choosiness, we wished to assess male mate choosiness with age over males' first 7 d of life. This period corresponds to males' realistic expected lifespan in the field (Rosewell & Shorrocks 1987). Third, we wished to test our deprivation hypothesis, which states that males deprived of females are less choosy than males that encounter females prior to the test (Dukas & Baxter 2014). Fourth, mate choice typically involves two-way interactions between prospective mates and their chooser. To focus on male mate choice, we always used females that consistently rejected males. Still there was a slight chance that females responded differently to young and mature males. To test this possibility, we conducted detailed observations quantifying females' responses to courting young and mature males. Finally, to help us explain the observed variation in mate choosiness with age, we wished to quantify relevant parameters in young and mature males. These included attractiveness to females, competitive ability and fertility.

## General Methods

We used wild-type *Drosophila melanogaster* housed in population cages with several hundred flies per cage. The cages were kept in an environmental chamber at 25°C and 60% relative humidity with a 12:12 h light:dark cycle, with the lights turning on at 10:00 a.m. Unless stated otherwise, we reared the experimental flies in 240-ml bottles with 50 ml of food and approx. 300 eggs per bottle. The food was a standard fly medium consisting of sucrose, cornmeal, yeast, agar and methyl paraben.

We sexed flies within 4 h of eclosion to ensure virginity. We used gentle aspiration to sex and transfer males individually into food vials, whereas we used CO<sub>2</sub> to sex and place females in groups of 20 per food vial. We did not use CO<sub>2</sub> with the focal males to avoid a possible confound owing to different recovery times for young than mature males. Each 40-ml vial contained 5 ml of the standard fly medium, and the females' vials also contained a dash of live yeast.

At the time of testing, mature males and females were 4 d old, young males were 1 d old, and virgin females were <20 h old and hence sexually immature. Immature virgin females are as sexually attractive to males as are sexually mature virgin females, but they always reject males' advances (Dukas & Dukas 2012). Using immature virgins, we ensured that all females persistently provided males with rejection signals, so we could focus on measuring a male's willingness to court a given female based on his motivational level and his subjective estimate of his likelihood of mating with that female. Recently mated females were 4 d old and mated with 4-d-old males 1–4 h before being used in the observation phases. In our laboratory, such females rarely remate (Dukas & Dukas 2012). We used small amounts of pink fluorescent powder to mark females to allow us to distinguish between the two female types when placed simultaneously in vials. Such marking has no effect on either fly behaviour or attractiveness (Dukas & Baxter 2014). Courtship observation phases were 15 min long, during which observers blind to male age recorded all courtship behaviours.

We used generalized estimating equations with gamma distributions and log link functions to analyse the data for which there were repeated measures for each male and generalized linear models with either gamma or Tweedie distributions with log link functions to analyse the data for which there was only one measure per individual using IBM SPSS (IBM-Corp. 2011). We applied sequential Bonferroni corrections to all post hoc comparisons.

### Effects of Male Age on Conspecific Mate Choosiness

First, we wished to broaden our investigation by examining the effect of male age on choosiness when encountering two conspecific females of distinct qualities. We tested two female qualities, body size (small vs. large), which is highly correlated with expected fertility in fruit flies as well as other insects (Robertson 1957; Honek 1993) and mating status (recently mated vs. virgin), which informs males about their likelihood of mating (lower in mated than in virgin females (Dukas 2005a)).

### Methods

In the first experiment, we tested whether there were age-related differences in males' mate choosiness between small and large females. We placed each male (young or mature) in a vial with one large and

one small virgin female and recorded the proportion of time he spent courting each female ( $n = 94$  males, half of each age).

We obtained small and large females by rearing flies under different densities. We reared small females in high density food vials with approx. 200 eggs per vial containing 1.5 ml of the standard fly medium, whereas we reared large females in low-density food bottles with approx. 75 eggs per bottle containing 50 ml of the standard fly medium. After testing, we measured the wing length of a random sample of 12 small and 12 large females. We removed the right wing of each female, mounted it on a microscope slide and measured the linear distance between the humeral–costal break and the end of the third longitudinal vein (Gilchrist & Partridge 1999). The wings of large females were approx. 45% longer than those of the small females,  $2.18 \pm 0.02$  vs.  $1.5 \pm 0.028$  mm, respectively (Wald  $\chi^2_1 = 328$ ,  $n = 24$ ,  $p < 0.001$ ).

In the second experiment, we tested for age-related differences in males' choosiness between virgin and recently mated females. We placed males in vials with one female of each type and recorded the proportion of time that males spent courting each female ( $n = 88$  males, half of each age).

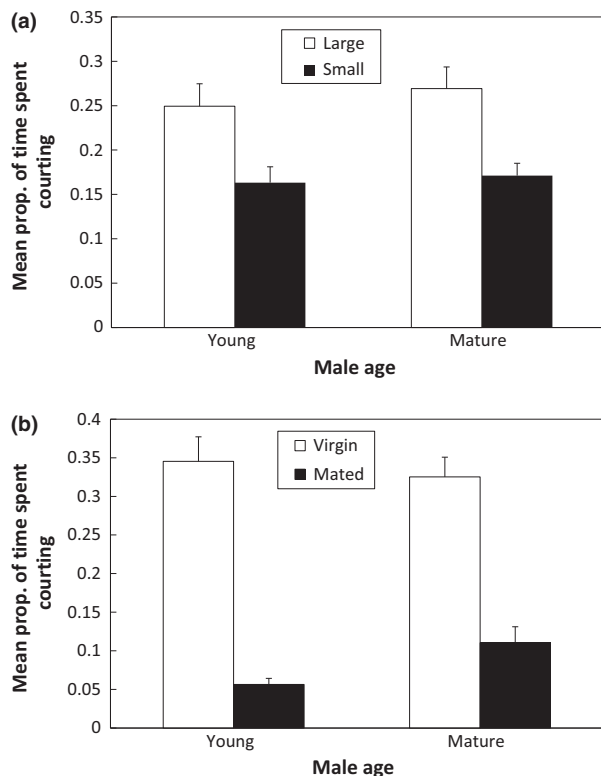
### Results

When each male encountered one small and one large female simultaneously, there was no interaction between male age and female type (Wald  $\chi^2_1 = 0.05$ ,  $n = 94$ ,  $p = 0.8$ ; Fig. 1a). Both male ages spent more time courting large than small females ( $p < 0.001$ ).

Males that were presented with both a virgin and recently mated female spent more time courting the former, but there was a significant interaction between male age and female category (Wald  $\chi^2_1 = 6.9$ ,  $n = 88$ ,  $p < 0.01$ ; Fig. 1b). While the young males spent less time courting the recently mated female than did the mature males ( $p < 0.05$ ), both male categories spent similar, large proportions of time courting the virgin females ( $p = 0.6$ ).

### Males' Mate Choosiness from Age 1–7 d

Our previous experiment as well as all the experiments in Dukas & Baxter (2014) compared mate choosiness in 1-d-old vs. 4-d-old males. Here, we wished to broaden our analysis to examine whether there is further decline in mate choosiness in males older than 4 d but within a realistic age range encountered in the field (Rosewell & Shorrocks 1987). Owing to enormous day and time of day



**Fig. 1:** The proportion of time ( $\bar{x} \pm 1$  SE) that young (1-d-old) and mature (4-d-old) males spent courting (a) large and small females ( $n = 47$  young and 47 mature males) or (b) virgin and mated females ( $n = 44$  young and 44 mature males) when presented with one female of each type simultaneously. While there was no significant ( $p = 0.8$ ) male age by female size interaction in (a), there was a significant interaction ( $p < 0.01$ ) between male age and female mating status in (b).

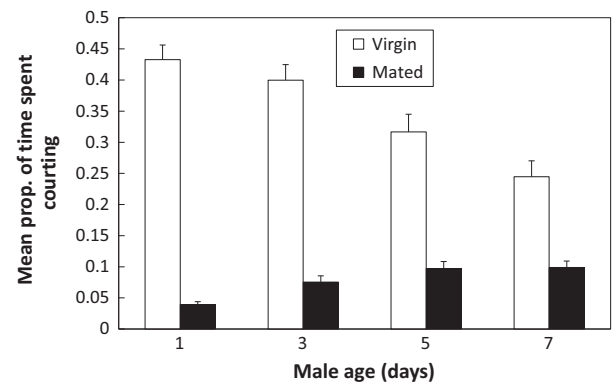
variation, we have to conduct all our comparisons simultaneously and thus focused on males that were 1, 3, 5 and 7 d old.

## Methods

We tested males that were 1, 3, 5 and 7 d old by placing them with one virgin and one recently mated female ( $n = 120$  males, 30 of each age) and recorded the proportion of time males spent courting each female.

## Results

While males of all ages spent more time courting virgin than recently mated females, there was a significant interaction between male age and female type. This was caused by both an increase with male age in the time spent courting recently mated females and a decrease with male age in the time spent courting



**Fig. 2:** The proportion of time ( $\bar{x} \pm 1$  SE) that males aged 1, 3, 5 and 7 d spent courting virgin and recently mated females when presented with one female of each type simultaneously ( $n = 120$  males, 30 of each age). There was a significant interaction ( $p < 0.001$ ) between male age and female mating status.

virgin females (Wald  $\chi^2_2 = 66$ ,  $n = 120$ ,  $p < 0.001$ ; Fig. 2).

## Effects of Female Deprivation on Male Mate Choosiness

The standard protocol in mate choice experiments is to sex eclosing adults and keep them in single-sex vials until the test to control for their experience and ensure female virginity. Males that encounter no females for a longer period might show lower mate-acceptance criteria, and this could explain our observed differences in mate choosiness between young and mature males (Dukas & Baxter 2014). To test for this possibility while controlling for male age, we compared mate choosiness of same-age, mature males that had either encountered and mated with females or encountered no females prior to the test.

## Methods

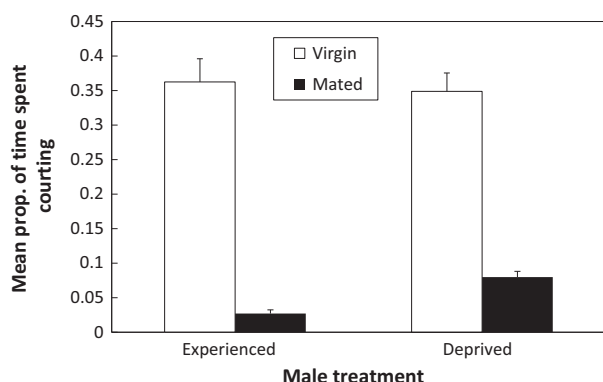
We randomly assigned males to either a deprived or an experienced treatment group. Deprived males encountered no females prior to the test while experienced males had a mature virgin female added to their vial on days 1, 2 and 3. To simulate realistic settings in which males experience both mature virgin and mated females, we kept all added females in the vials. We also moved all flies into new food vials on Day 3 to ensure the availability of fresh food. On Day 4, we moved the males into test vials with one virgin female and one recently mated female and recorded the proportion of time that the males spent courting each female ( $n = 72$  males, half from each treatment).

## Results

There was a significant interaction between male treatment and female type (Wald  $\chi^2_2 = 21$ ,  $n = 72$ ,  $p < 0.001$ ; Fig. 3). Both experienced and deprived males courted virgin females for similar proportions of time ( $p = 0.75$ ). However, experienced males courted mated females for significantly less time than deprived males did ( $p < 0.001$ ).

### Females' Responses to Courtship by Young vs. Mature Males

Male fruit flies that encounter a female have a variety of cues that can inform them about their likelihood of mating with that female. First, as in many insects, the female's blend of cuticular hydrocarbons contains species- and age-specific information (Jallon 1984; Fervour 2005; Howard & Blomquist 2005; Everaerts et al. 2010). Second, at least two volatiles provided by males during copulation, *cis* vaccenyl acetate (cVA) and CH503, mark the female as mated and dramatically reduce that female's attractiveness to males (Brieger & Butterworth 1970; Zawistowski & Richmond 1986; Yew et al. 2009; Ng et al. 2014). Finally, if the male initiates courtship, the female's behaviour might provide further information about her receptivity (Spieth 1952; Lasbleiz et al. 2006). Although we used females that rejected males, one might argue that females could vary in their responses towards young and mature males and that this in turn determined the observed difference in male mate choosiness. To assess this possibility, we compared the behavioural responses of mature virgin females to young and mature males.



**Fig. 3:** The proportion of time ( $\bar{x} \pm 1$  SE) that mature (4-d-old) males experienced with females ( $n = 36$ ) and mature males deprived of females ( $n = 36$ ) spent courting virgin and recently mated females when presented with one female of each type simultaneously. There was a significant interaction ( $p < 0.001$ ) between male age and female mating status.

## Methods

Our methods were identical to those used in a project dedicated to quantifying female behavioural responses to males, in which we have found significant variation in the behaviour of different female categories towards conspecific and heterospecific males (R. Dukas, unpubl. data). We placed four sexually mature virgin females approx. 25 h old inside a  $10 \times 10 \times 10$  cm Plexiglas cage. The cage contained a cylinder of 5 ml regular food medium with a dash of live yeast illuminated with light from an LED lamp. We then added a single male, either 1 d old or 4 d old. We recorded male and female behaviour for 5 min starting with the first courtship, which usually occurred within a few min. We recorded all starts and ends of male courtship bouts and the female's behaviour while being courted. Females either were non-responsive and appeared to continue with their pre-courtship activity, most often feeding, or clearly responded to males with wing fluttering, raising their abdomen or decamping (Spieth 1952). While we also recorded whether females were feeding, walking or idling on the cage wall, we had decided *a priori* to focus on the three explicit female rejection behaviours. We calculated, for each female, the proportion of time spent wing fluttering and abdomen raising, and the decamping rate. We calculated the proportions of times and decamping rates based on the trial duration for each female, which was 5 min in trials with no matings and the mating latency in trials with matings. We tested 20 males of each age category.

## Results

Females' rates of abdomen raising ( $1.1 \pm 0.5$  vs.  $0.65 \pm 0.37$  per min, Wald  $\chi^2_2 = 1.1$ ,  $n = 40$ ,  $p = 0.3$ ), wing fluttering ( $4 \pm 1.2$  vs.  $3.4 \pm 1$  per min, Wald  $\chi^2_2 = 0.9$ ,  $n = 40$ ,  $p = 0.6$ ) and decamping ( $0.26 \pm 0.08$  vs.  $0.37 \pm 0.15$  per min, Wald  $\chi^2_2 = 1.5$ ,  $n = 40$ ,  $p = 0.2$ ) were similar towards young and mature males. Sixty per cent of the young and 60% of the mature males mated during the 5-min trials, and their mating latencies were not significantly different ( $69 \pm 12$  vs.  $100 \pm 25$  for young and mature males, respectively, Wald  $\chi^2_2 = 1.1$ ,  $n = 24$ ,  $p = 0.3$ ).

### Attractiveness to Females of Young and Mature Males

A variety of female and male parameters could influence the observed age-specific variation in male mate choosiness. We began by assessing the per-



ceived attractiveness of males to females. As young and mature males courted with similar intensities and mated at similar frequencies in no-choice trials with mature virgin females (Dukas & Baxter 2014), we focused on the mating latencies, which we expected to be negatively correlated with male attractiveness.

## Methods

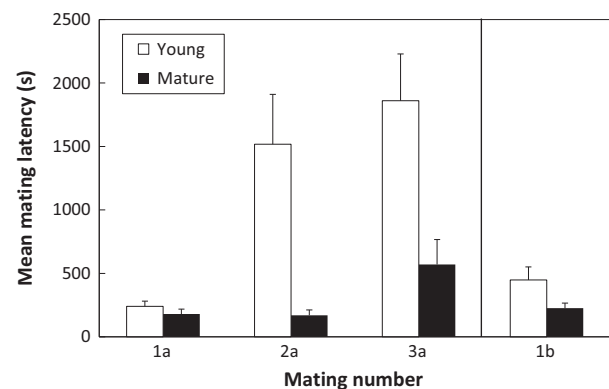
We tested whether young and mature males differed in their mating latencies across three successive matings. We placed each male inside a food vial with a mature female and allowed them to mate ( $n = 32$  males, half of each age). If males did not mate with the female they were originally presented with in each trial, we replaced the female with a new one every 20 min until mating occurred, or until the 60-min trial was over. Observers blind to male age recorded each male's mating latency. After each mating, we gave the males a 15-min break before placing them with a new female. All of the mature males mated in each of the three trials. In the first trial, all of the young males also mated. In the second trial, two young males did not mate within the hour and were given extra time to mate. In the third trial, four of the 16 young males did not mate and these matings were excluded from the analysis. In a follow-up experiment, we compared the mating latencies of young and mature males only in their first mating ( $n = 42$  males, half of each age).

## Results

There was a significant male age by trial interaction for mating latencies (Wald  $\chi^2_1 = 22$ ,  $n = 32$ ,  $p < 0.001$ ; Fig. 4, results shown to the left of the black line). The mating latencies of young and mature males were similar in the first mating trial ( $p = 0.9$ ), but mating latencies were shorter in mature than young males in the subsequent trials (Wald  $\chi^2_1 = 23$ ,  $n = 32$ ,  $p < 0.001$ ). In the follow-up experiment, mature males had shorter mating latencies than young males in their first mating (Wald  $\chi^2_1 = 6.6$ ,  $n = 42$ ,  $p = 0.01$ ; Fig. 4, results shown to the right of the black line).

## Competitive Ability of Young and Mature Males

As the operational sex ratio in fruit flies is strongly male biased (Bateman 1948; Spieth 1974), one can readily observe two males pursuing the same female



**Fig. 4:** The mating latencies ( $\bar{x} \pm 1$  SE) of young (1-d-old) and mature (4-d-old) males across three successive mating trials. Mating numbers 1a, 2a and 2a correspond to mating latencies in the first experiment ( $n = 16$  young and 16 mature males; significant male age by trial interaction ( $p < 0.001$ ), whereas mating number 1b corresponds to results from the follow-up experiment ( $n = 21$  young and 21 mature males with shorter mating latencies by mature than young males,  $p = 0.01$ ).

simultaneously. We thus wished to measure the competitive ability of young and mature males placed together with a single female. Specifically, we wanted to see whether males of a given age could monopolize the female and thus reduce the mating probability of males of the other age.

## Methods

We conducted two types of trials. In the individual trials, we placed one male (young or mature) in a vial with one immature virgin female ( $n = 54$  males). In the competitive trials, we placed both a young and mature male in a vial with an immature virgin female ( $n = 50$  males). We coloured one male per vial in the competitive trials with a pink fluorescent powder to allow the observers to differentiate between them. Male colouring was counterbalanced across trials and did not affect male courtship (Wald  $\chi^2_1 = 0.1$ ,  $n = 50$ ,  $p = 0.7$ ).

## Results

In the individual trials, when each male was alone with a virgin female, both young and mature males spent similar proportions of time courting (Wald  $\chi^2_1 = 0.001$ ,  $n = 54$ ,  $p = 1$ ; Fig. 5). However, in the competitive trials, when a young and mature male were together with a virgin female, mature males spent significantly greater proportions of time courting than did young males (Wald  $\chi^2_1 = 9.6$ ,  $n = 50$ ,  $p < 0.005$ ; Fig. 5).

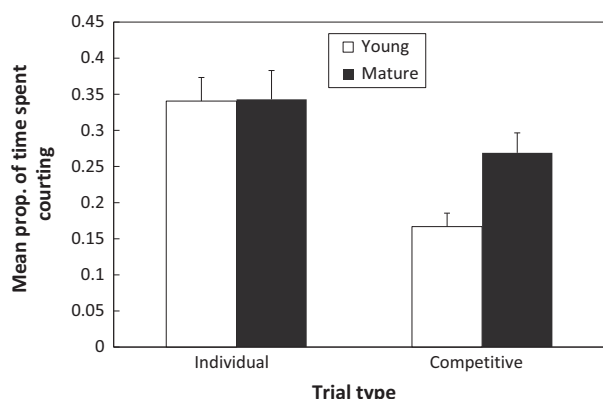
### Fertility of Young and Mature Males

Our previous data (Dukas & Baxter 2014) indicated similar fertility of young and mature males mated once with virgin females. The extensive literature on males' sophisticated sperm allocation strategies (Wedell et al. 2002; Lüpold et al. 2011) suggests, however, that mate choosiness can be higher in males with more limited supplies of sperm and seminal fluids than in less limited males. We thus wished to quantify the total fertility of males over three successive matings. As male fertility is determined by both sperm and seminal fluid, we preferred to count male offspring rather than sperm. Furthermore, we counted adult offspring because they are a more relevant measure of male fitness than fertilized eggs.

### Methods

We allowed young ( $n = 16$ ) and mature ( $n = 16$ ) males to mate three times in succession with mature virgin females. We reported the mating latencies for these males above (Fig. 4, left side). We transferred the mated females into fresh food vials with live yeast each day until they no longer laid fertilized eggs. Observers blind to male treatment counted all adult offspring. We excluded from the analysis 13 females (14%) that died on or before Day 5 of egg laying.

In a follow-up experiment, we allowed young and mature males to mate three times in succession with three virgin 3-d-old females and counted the offspring that the males fathered in their third mating. Although we started with 22 males of each age

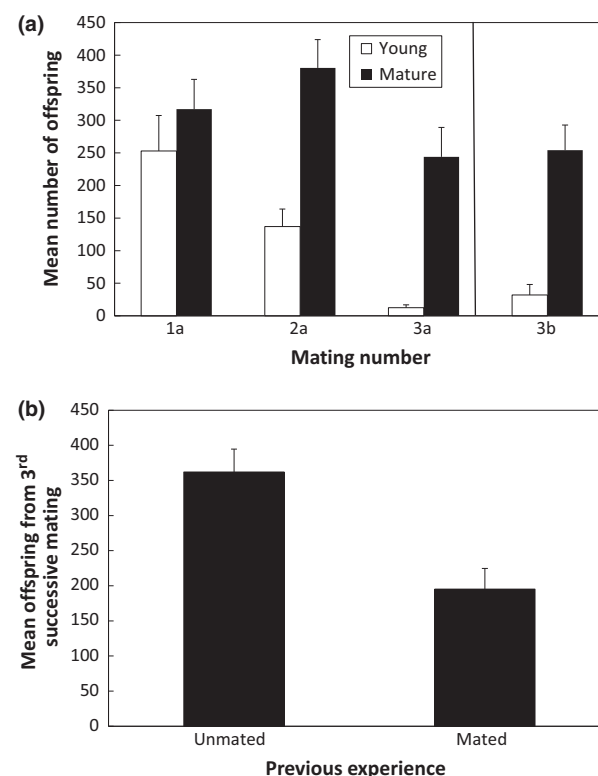


**Fig. 5:** The proportion of time ( $\bar{x} \pm 1$  SE) that young (1-d-old) and mature (4-d-old) males spent courting virgin females under individual (one male per vial,  $n = 54$  males) and competitive (one young and 1 mature male per vial,  $n = 50$  males) conditions. Mature males spent more time than young males courting females under the competitive than individual condition,  $p < 0.005$ .

category, 50% of the young males and 5% of the mature males failed to mate for the third time (Pearson  $\chi^2_1 = 11.5$ ,  $n = 44$ ,  $p < 0.001$ ), leaving us with sample sizes of 11 and 21, respectively.

### Results

There was a significant male age by mating number interaction for fertility (Wald  $\chi^2_2 = 58$ ,  $n = 32$  males,  $p < 0.001$ ; Fig. 6a, results shown to the left of the black vertical line). Young and mature males fathered similar numbers of offspring in their first mating ( $p = 1$ ). Mature males, however, fathered significantly more offspring than young males in their second and third matings (both  $p < 0.001$ ). Overall, mature males fathered more than twice as many offspring as did young males ( $p < 0.001$ ).



**Fig. 6:** (a) The number of offspring ( $\bar{x} \pm 1$  SE) fathered by young (1-d-old) and mature (4-d-old) males across three successive mating trials. Mating numbers 1a, 2a and 3a correspond to offspring produced from the first experiment ( $n = 16$  young and 16 mature males), whereas mating number 3b corresponds to results from the follow-up experiment ( $n = 11$  young and 21 mature males). Mature males fathered more offspring in all matings ( $p < 0.001$ ) except for 1a ( $p = 1$ ). (b) The number of offspring ( $\bar{x} \pm 1$  SE) fathered by previously unmated ( $n = 19$ ) and previously mated ( $n = 20$ ) mature (4-d-old) males as a result of their third consecutive mating. Unmated males fathered more offspring than previously mated males ( $p < 0.02$ ).

In the follow-up experiment, mature males again fathered more offspring in their third successive mating than did young males (Wald  $\chi^2_1 = 17$ ,  $n = 32$ ,  $p < 0.001$ ; Fig. 6a, results shown to the right of the black vertical line).

### Fertility of Mature Males That Have Either Encountered or Been Deprived of Females

As we documented higher mate choosiness in males that encountered females than in males deprived of females, we wished to examine whether this difference could be attributed to lower sperm and seminal fluid supplies in the former males. We thus compared their fertility over three successive matings.

### Methods

The mated treatment ( $n = 20$ ) consisted of males that had mated once each day on days 1–3. The unmated males ( $n = 20$ ) had not mated prior to the test. On Day 4, we allowed all males to mate successively with three females and kept females from the third matings for progeny count as described above. We excluded from the analysis one female that died on Day 4 of egg laying.

### Results

Previously unmated mature males produced significantly more offspring in their third consecutive mating than did previously mated males (Wald  $\chi^2_1 = 6$ ,  $n = 39$ ,  $p = 0.014$ ; Fig. 6b).

### Discussion

Our two tests of the effects of male age on conspecific mate choosiness showed no effect of age on males' preference to court large over small females (Fig. 1a) but higher choosiness by young than mature males encountering virgin and mated females (Fig. 1b). Together with our results showing higher mate choosiness in young than mature males encountering conspecific and closely related heterospecific females (Dukas & Baxter 2014), we believe that the overall pattern is of young males being more reluctant than mature males to persist in courting females with whom the probability of mating is low. That is, males have some knowledge about their relatively low likelihood of mating with mated conspecific females and virgin heterospecific females (Brieger & Butterworth 1970; Jallon 1984; Ferveur 2005; Ng et al. 2014) as indicated by the fact that, regardless of age, males

spend less time courting such females (Figs 1–3 and Dukas & Baxter 2014). The virgin conspecific females, however, are highly attractive and even small females appear to be above a threshold attractiveness that elicits as much courtship from young as from mature males.

Refining our analysis of male age and mate choosiness, we found a steady pattern of reduction in mate choosiness with males' age (Fig. 2). This pattern is consistent with the two non-mutually exclusive hypotheses on the effect of an individual's age on mate choosiness. First, from a life history perspective, one would expect a positive correlation between the current mate choosiness and the expected future mating opportunities. That is, older males face a shorter time horizon due to senescence and thus should be less choosy. Field data indicate expected lifespan of approx. 7 d in fruit flies (*D. melanogaster*) (Rosewell & Shorrocks 1987), and male fertility shows dramatic decline in males over 7 d old (Edward & Chapman 2013), so the reduced mate choosiness with male age strongly agrees with life history theory, which has traditionally focused on offspring production by females (Stearns 1992). Indeed, data from distinct taxa indicate reduced mate choosiness with age in females (Kodric-Brown & Nicoletto 2001; Moore & Moore 2001; Uetz & Norton 2007; Wilgers & Hebets 2012). While we focus here on the ultimate mechanism for the reduction in mate choosiness with age, a possible related proximate mechanism could be a reduction in males' perceptual ability to discriminate between female types with age. That is, the mechanism for the age-specific reduction in mate choosiness may be based on differences in either perception or decision.

The other hypothesis linking age and mate choosiness involves the fact that, often, age is positively correlated with the length of deprivation from the other sex. Both intuition and optimality models predict a positive correlation between the encounter rate with preferred prospective mates and mate choosiness (Wilson & Hedrick 1982) and data for females in a variety of species agree with this prediction (Shelly & Bailey 1992; Berglund 1995; Dukas 2005b; Willis et al. 2011). Hence, in our protocols, encountering no females at all for longer durations may have decreased male mate choosiness. We critically tested a prediction derived from this hypothesis, that mate choosiness would be higher in mature males previously exposed to females than in same-age, mature males previously deprived of females. Our results indeed agree with this prediction (Fig. 3), but we cannot conclude whether mate choosiness increases due to the daily matings by the experienced males or their learning to



focus courtship on females that are more likely to accept them as mates. Males' learning in the context of courtship has been well examined in this system, so we know that, even among mature males deprived of matings, males that have experienced rejections by either heterospecific or mated females are more choosy than inexperienced males (Dukas 2005a; Dukas & Dukas 2012). It is likely though that both the effects of deprivation and learning play a role in male mate choosiness.

In addition to the two hypotheses linking age and mate choosiness discussed above, one can readily think of other age-related factors that can influence male mate choosiness. These include a positive correlation between males' mate choosiness and both their own age-specific attractiveness to females and ability to outcompete other males, and a negative correlation between males' age-specific sperm and seminal fluid stores and mate choosiness (Fawcett & Johnstone 2003; Byrne & Rice 2006). Our experiments addressing these factors revealed that young males are less attractive to females than mature males as indicated by the longer time it took females under no-choice conditions to accept young than mature males as mates. We found such differences even in males' first encounters with females, and the gap in attractiveness widened dramatically in males previously mated once or twice (Fig. 4). When we allowed one young and one mature male to compete for a single female, the mature males monopolized the female, indicating that they can outcompete young males for access to females (Fig. 5). Finally, our fertility tests indicated that mature males are much more fertile than young males (Fig. 6a).

Previous studies also documented higher mating success of older over younger males. In one set of experiments involving two males of distinct ages and a single female (Long et al. 1980), males' mating success was highest when 8 d old, intermediate when 4 d old and lowest when 2 d old. Male fertility in that study was low when males were 2 d old and equally higher when they were 4 and 8 d old. In our previous study (Dukas & Baxter 2014), we found a higher mating success of mature, 4-d-old males than of young, 1-d-old males when we placed one male of each age with a single female. Our new data indicate that this outcome could reflect both the higher competitive ability of the mature males (Fig. 5) and females' preference for mature over young males (Fig. 4). While our data agree with those of Long et al. (1980) indicating higher fertility of mature than young males, we found no difference in males' fertility in their initial mating (Dukas & Baxter 2014) but much lower

fertility in subsequent matings (Fig. 6a). Intriguingly, males' fertility, as measured in their third successive mating, was lower in males exposed to females prior to the test (Fig. 6b). Such males could mate with virgin females only once per day prior to the test day. The fact that this was sufficient to reduce male fertility indicates that the males are rather limited in their ability to mate successively and that such limitation could drive high male mate choosiness.

Surprisingly, two of the three male characteristics that we measured suggest that young males should actually be less choosy than mature males because we expect less attractive and less competitive males to be more willing to court less attractive females. There are two non-mutually exclusive explanations to this apparent contradiction. First, it is possible that the males' relatively low sperm and seminal fluid stores override the other factors and reduce their willingness to persist in courting less attractive females. Second, the young males' higher expected probability of encountering receptive females, perhaps combined with multiple costs of courtship and mating, may make them more choosy than mature males.

Overall, our data show that, in males with no previous exposure to females, there is a gradual decrease in male mate choosiness with age (Fig. 2). Prior exposure to conspecific females, however, significantly increases male mate choosiness (Fig. 3). Furthermore, low expected fertility, most likely due to ejaculate limitation (Fig. 6), is associated with high levels of male mate choosiness. Finally, previous work indicates that male learning, based on either encounters with conspecific or heterospecific females, increases male mate choosiness (Dukas 2004, 2005a; Dukas & Dukas 2012). Combining all this information, we expect that, in nature, young males will be choosy and that they will maintain high levels of choosiness once they gain further experience with age. We still do not know whether conflicting factors, most notably, decreasing probability of future matings with increasing age, would ultimately reduce male mate choosiness. Our data thus suggest that male mate choosiness may be an important factor influencing both sexual selection and incipient speciation.

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