

Original Article

Mate choosiness in young male fruit flies

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Mate choice by males has been documented in many taxa including fruit flies (*Drosophila* spp.). It is still unclear, however, whether male mate choice varies much with age and one cannot readily predict the direction of change in male choosiness with age. We compared mate choice in young (1 day old) and mature (4 days old) male fruit flies (*Drosophila melanogaster*). Young males spent much less time courting heterospecific females than did mature males, but both male categories spent similar time courting conspecific females. We obtained similar results indicating higher selectivity of young than mature males in descendants of wild-caught flies, when males had an opportunity to learn to avoid heterospecific females and when males had a choice between courting conspecific and heterospecific females. The common methodology in the discipline has been to let virgin flies age for at least a few days prior to their use in experiments. Our experiments, which employed protocols sensitive to male age and experience, suggest that male fruit flies may be more choosy than previously thought and hence can contribute much more to sexual selection and incipient speciation than previously appreciated.

Key words: age, courtship, *Drosophila melanogaster*, fruit flies, learning, male mate choice.

INTRODUCTION

The past decade has seen 2 fundamental shifts in our understanding of male sexual behavior, which may have broad implications for research on sexual selection and reproductive isolation. First, there is a growing appreciation that mate choice by males is prevalent even when males provide only sperm and that males gain from being choosy. Examples for female traits preferred by males include virginity, sexual receptivity, larger body size, and enhanced ornamentation (Bonduriansky 2001; Gowaty et al. 2003; Clutton-Brock 2009; Edward and Chapman 2011, 2012). Second, males can learn to refine their mate choice criteria and courtship behavior based on feedback from females (Dukas 2006; Servedio and Dukas 2013). Examples include male fruit flies (*Drosophila melanogaster*, *Drosophila pseudoobscura*, and *Drosophila persimilis*) (Dukas 2004, 2009; Ellis and Carney 2009; Dukas and Dukas 2012), Trinidadian guppies (*Poecilia reticulata*) (Magurran and Ramnarine 2004), and sticklebacks (*Gasterosteus* spp.) (Kozak and Boughman 2009).

Although male mate choice has been documented in numerous taxa, the role of male age in his mate choosiness is not well studied. Existing theory and data could lead us to conflicting predictions about the effect of male age on male mate choice. Because young males often have a mating disadvantage (e.g., Long et al. 1980), one could argue that young males would be more willing to court lower quality females than would older males. Young males

might also have an exploration phase, during which they approach a wide variety of females in order to gain experience, which they could later use to optimize their choice of courtship targets (Dukas et al. 2006; Dukas and Dukas 2012). On the other hand, one can apply basic economic considerations to predict that, under the typical experimental settings where males cannot mate prior to the test, older males, who experience a longer period with no matings, would employ a lower female acceptance threshold than would younger males (Wilson and Hedrick 1982).

The common practice in fruit fly research on mate choice is to separate the sexes on eclosion and maintain them in single-sex vials for 4–8 days to control for their experience and to ensure virginity and sexual maturity by the time of testing (e.g., Noor 1995). Neurogenetic studies on courtship learning also report best results with 5-day-old males (Connolly and Tully 1998, p. 270). Following the tradition in multiple disciplines, we also focused our research on 4-day-old males (Dukas 2004, 2008; Dukas and Dukas 2012) but recently realized that we know little about the sexual behavior of males much younger than 4 days, although the males reach sexual maturity when they are less than 1 day old (Strömnes and Kvelland 1962). Male fruit flies (*D. melanogaster*) have been shown to express mate choice (Byrne and Rice 2006) from which they might attain small fitness gain (Edward and Chapman 2012), at least when they are 2–7 days old (Edward and Chapman 2013).

To clarify the issue of male mate choosiness as a function of age, we compared the levels of courtship toward conspecific and heterospecific (*Drosophila simulans*) females and degree of learning to

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avoid heterospecific females with experience in young (1 day old) and mature (4 days old) male fruit flies (*D. melanogaster*).

METHODS

General

We primarily used vigorous laboratory populations of Canton S *D. melanogaster* and *D. simulans* (collected in Southern California in 2009) following standard protocol (Dukas 2010; Dukas and Dukas 2012). We replicated key results with descendents of wild *D. melanogaster* caught in Southern Ontario several months prior to the experiments. We sexed by aspiration 1- to 4-h-old males and kept them individually in regular 40-mL vials (25 mm in diameter and 95 mm long) each containing 5 mL standard food. Young males were 1 day old and mature males were 4 days old. All females were virgin. Observers blind to fly identity conducted all the observations. Unless noted otherwise, we analyzed the data with a generalized linear model with gamma distribution and log link function using IBM SPSS (IBM Corp. 2011).

Courtship of and learning about heterospecific females

In experiment 1, we tested for the tendency of Canton S males to court heterospecific females and their ability to learn to avoid courting such females (Dukas 2004, 2005a; Dukas and Dukas 2012). The protocol (Table 1) included 2 male ages (young and mature) and 2 experience treatments (experienced and inexperienced). After the experience phase, we tested males with 2 female types (heterospecific or conspecific females).

In the experience phase, we placed males of the experienced treatment each with two 4-day-old heterospecific females and males of the inexperienced treatment each alone in vials. At the end of the 30-min experience phase, we moved all males into fresh vials. After a 15-min break, we started the test phase. We placed half the males of the experienced treatment and half the males of the inexperienced treatment each with 2 heterospecific females and the other half of males of each treatment each with 2 immature conspecific females. We used immature conspecific females younger than 20 h so that all males were rejected by the females. We recorded all courtship bouts during the 15-min test trials and then calculated for each male the proportion of time spent courting (Dukas and Mooers 2003; Dukas 2004). In a follow-up experiment, we compared courtship by inexperienced young and mature males from the wild population. We placed each male either with 2

Table 1
Protocol used in experiment 1

Male age	Male experience	Test female
Young	Heterospecific females	Heterospecific females
Young	Heterospecific females	Conspecific females
Young	None	Heterospecific females
Young	None	Conspecific females
Mature	Heterospecific females	Heterospecific females
Mature	Heterospecific females	Conspecific females
Mature	None	Heterospecific females
Mature	None	Conspecific females

Males were either young (1 day old) or mature (4 days old). They either experienced rejection by heterospecific females during the experience phase or were inexperienced, and they encountered either heterospecific or conspecific females in the test.

heterospecific females or with 2 immature conspecific females and recorded all courtship bouts during the 15-min test trials.

Male mate choice

In experiment 2, we tested whether males that encounter conspecific and heterospecific females simultaneously also show age-related differences in selective courtship. We placed each male with 1 heterospecific female and 1 immature conspecific female and recorded the total time males spent courting each female. We analyzed the data with a generalized estimating equation with female species as a repeated measure within each male (IBM Corp. 2011).

Mating success and fertility

In experiment 3, we assessed the mating success and fertility of young and mature males. We placed each male individually with a single 4-day-old conspecific female and counted the number of males mated within 1 h. We then transferred a subset of 20 females that mated with young males and 20 females that mated with mature males individually into regular food vials and transferred them daily until they stopped laying fertilized eggs. We counted all emerging adult progeny. We also quantified male fertility in flies of the wild population.

In experiment 4, we quantified the mating success of young and mature males competing for a single 4-day-old conspecific female. We placed each female with 1 young and 1 mature male. To identify the males, we marked 1 male in each trial with small amounts of visible, pink fluorescent powder (Crumpacker 1974). We marked young males in half the trials and mature males in the other half. Marking had no effect on mating success (chi-square test, $\chi^2_1 = 0.65$, $n = 200$, $P = 0.4$).

RESULTS

Courtship of and learning about heterospecific females

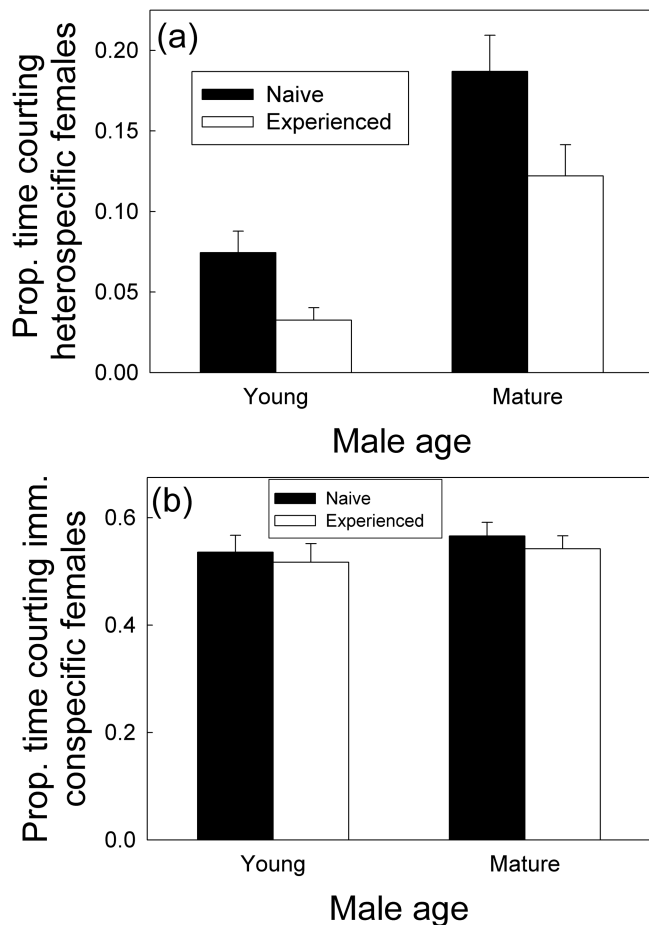
The young males spent less time courting heterospecific females than did the mature males regardless of experience (Wald $\chi^2_1 = 39.4$, $n = 192$, $P < 0.001$; Figure 1a). Both male categories, however, spent similar proportions of time courting conspecific females (Wald $\chi^2_1 = 0.5$, $n = 192$, $P = 0.47$; Figure 1b) and showed a similar reduction in courting heterospecific females with experience (Wald $\chi^2_1 = 12.3$, $n = 192$, $P < 0.001$ for the effect of experience; Wald $\chi^2_1 = 1.2$, $n = 192$, $P = 0.26$ for the male age). In the wild flies, young males also courted heterospecific but not conspecific females much less than mature males (Wald $\chi^2_1 = 8.5$, $n = 128$, $P < 0.005$ for male age by female interaction; Figure 2a).

Male mate choice

When given a choice between courting conspecific and heterospecific females, the young males spent less time courting the heterospecific females than the mature males did. The 2 male categories, however, spent similar proportions of time courting the conspecific females (Wald $\chi^2_1 = 17$, $n = 60$, $P < 0.001$ for male age by female interaction; Figure 2b).

Mating success and fertility

In experiment 3, when each conspecific female was placed with a single male, the young and mature males mated at similar frequencies (95% and 92%, respectively, $\chi^2_1 = 0.27$, $n = 46$, $P = 0.6$). There was no difference in progeny number between young and

**Figure 1**

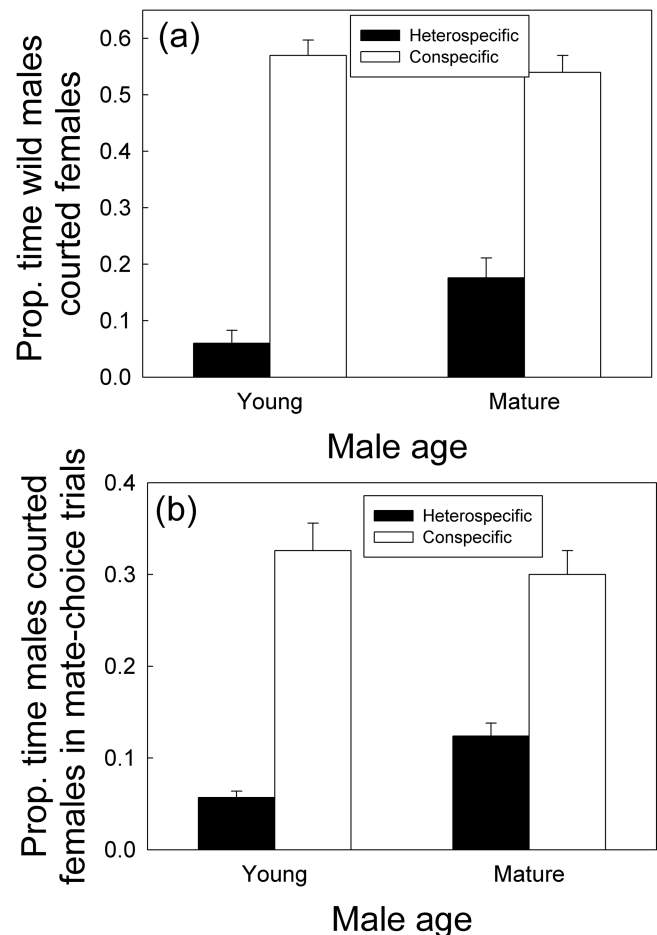
The proportion of time (mean \pm 1 SE) young (1 day old) and mature (4 days old) males spent courting (a) heterospecific or (b) immature conspecific females. Males either were inexperienced with females (black bars) or had experienced rejection by heterospecific females (white bars). $n = 384$ focal males.

mature males (mean \pm SE: 382 ± 34 vs. 259 ± 35 , respectively, Wald $\chi^2_1 = 2.7$, $n = 40$, $P = 0.1$). In the fertility test with the wild flies, young and mature males fathered a similar number of progeny (299 ± 56 vs. 261 ± 47 , respectively, Wald $\chi^2_1 = 0.1$, $n = 18$, $P = 0.7$).

In experiment 4, when each conspecific female was placed with 1 male of each age, the mating frequency of mature males was more than 3 times higher than that of young males (77.5% vs. 22.5%, respectively, $\chi^2_1 = 57$, $n = 200$, $P < 0.001$).

DISCUSSION

Our results indicate that, soon after reaching sexual maturity, male fruit flies are rather choosy in their female choice. We found these results in 2 lines of flies (Figures 1a and 2a), when males were presented with either 1 (Figures 1a and 2a) or 2 female species simultaneously (Figure 2b) and when males had prior experience with being rejected by heterospecific females (Figure 1a). Overall, inexperienced young males (1 day old) were more than twice as selective in their courtship as inexperienced mature males (4 days old) and experienced young males were almost 6 times more selective than inexperienced mature males. Although the effect of age is novel, we have previously demonstrated the effects of experience

**Figure 2**

(a) The proportion (mean \pm 1 SE) of time young (1 day old) and mature (4 days old) wild males ($n = 128$) spent courting heterospecific and immature conspecific females. (b) The proportion of time (mean \pm 1 SE) young (1 day old) and mature (4 days old) males ($n = 60$) given a choice between heterospecific and immature conspecific females spent courting each female.

(Dukas 2004, 2008; Dukas and Dukas 2012). Nevertheless, it is important to know that young males learn as well as mature males in the context of mate choice, even though they may be less likely to be accepted by sexually mature conspecific females when competing with mature males (experiment 4). Although there is now extensive literature on mate choice by males (Bonduriansky 2001; Edward and Chapman 2011), we know of no other study relating male age to mate choosiness. Edward and Chapman (2013) reported fitness benefit from mate choice in males 2–7 days old, but we cannot readily relate their result to ours because we compared 1- and 4-day-old males.

It is likely that females preferred mature over young males in experiment 4 owing to more attractive body odors (Arienti et al. 2010). Although we did not observe direct male–male interference, we cannot rule out subtle interactions. We should note that we used in experiments 1 and 2 females that rejected males' advances, so female receptivity to males could not confound our male choice results (Figures 1 and 2). We cannot rule out the possibility that females responded differently to young and mature males. Our previous research, however, indicates that males relentlessly court unreceptive, immature females, which the males find highly attractive, in spite of the persistent rejections by and zero mating success with

such females (Dukas and Dukas 2012). Another possibility is that young males are not fully sexually mature and thus require stronger stimulation for initiating courtship. This mechanistic explanation does not negate the ultimate outcome of greater choosiness by young than mature males.

It appears that young males rely on innate biases to show rather high levels of discrimination toward prospective mates based on the distinct cuticular hydrocarbons of conspecific and heterospecific females (Jallon and David 1987; Cobb and Jallon 1990). They then quickly show further increase in their choosiness based on experience. It is thus likely that the baseline level of male choosiness in nature would be rather high in young males and that it would increase even further with experience. It is even possible that males that continuously encounter a variety of females since eclosion might be very choosy by the time they are 4 days old. This possibility requires critical evaluation.

It is possible that the lower levels of mate choosiness by mature males in the current report and numerous others (Streisinger 1948; Noor 1996; Dukas 2004) were caused by a reduction in the acceptance threshold of males who have not encountered females for at least a few days. Although the idea of low levels of selectivity owing to “sexual excitement” by female-deprived males appeared early in the literature, it was not backed by data (Mayr and Dobzhansky 1945, p. 80). However, a few reports support the basic theoretical expectation (Wilson and Hedrick 1982) that encounter rates with preferred prospective mates would be positively correlated with the mate-acceptance threshold (Shelly and Bailey 1992; Berglund 1995; Dukas 2005b; Willis et al. 2011).

Our results suggest that male fruit flies can contribute much more to sexual selection and incipient speciation than previously thought. Future experimental research should thus carefully consider male early experience, and theoretical models ought to give further consideration to males’ contributions to incipient speciation and sexual selection.

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