Teneral matings in fruit flies: male coercion and female response

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Extensive research indicates that sexually mature female fruit flies typically choose with whom to mate, a process that is crucial for the operation of sexual selection and population divergence via female choice. We followed up on field data suggesting that male fruit flies (*Drosophila melanogaster*) force copulate with teneral females, which are recently eclosed females characterized by their folded wings and soft, light coloured bodies. Our results indicated that males succeeded in mating with about 20% of the teneral females from our Canton-S population and 15% of the teneral females from a local, wild-caught *D. melanogaster* population. Males were most likely to mate with recently eclosed females and failed to mate with females older than 1 h posteclosion. Close behavioural observations indicated coercion by the males and resistance by the teneral females. The teneral females continued to fight off males throughout copulation and this probably contributed to the shorter mating duration of teneral females relative to that of mature females. Although teneral females failed to foil some forced copulation attempts, they could reduce the impact of such copulations by subsequently remating with a male of their choosing. Indeed, tenerally mated females were six times more likely to remate than were control females that had mated when mature. Our results substantiate a novel aspect of sexual conflict in fruit flies, which could counteract processes driven by female mate choice.

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Fruit flies (*Drosophila* spp.) have been a central model system in research on sexual selection and speciation (Coyne & Orr 2004; Mallet 2006; Noor & Feder 2006; Ritchie 2007; Pitnick & Hosken 2010; Rundle & Boughman 2010). Research with fruit flies (*D. melanogaster*) indicates that the females can choose with whom to mate and otherwise deflect males' persistent advances (Spieth & Ringo 1983). A notable exception is the intriguing report by Markow (2000) indicating that males apparently forced copulation with teneral females in wild populations of *D. melanogaster* and *D. simulans*. The males approached teneral females, readily identified by their light colour, soft body and folded wings, and mounted them following either brief courtship or no courtship at all. The teneral females showed no obvious rejection behaviour (Markow 2000). Surprisingly, we know of no follow-up studies elaborating on Markow's (2000) observations in spite of their potential importance.

The general topic of sexual conflict has received significant attention (reviewed in: Arnqvist & Rowe 2005; Fricke et al. 2010) and the specific issue of forced copulation has been examined in a variety of taxa (McKinney et al. 1983; Clutton-Brock & Parker 1995; Thornhill & Palmer 2000). Still, there have been few detailed empirical studies examining male and female decisions associated with forced copulation. A notable exception is work on scorpion flies (*Panorpa* spp.), in which legitimate matings involve male nuptial gifts to females and forced matings consist of males with no food using coercion countered with strong female resistance (Thornhill 1980, 1984). If teneral matings are a regular occurrence in fruit flies, and if they indeed constitute forced copulations, fruit flies can be used as a model system for research on forced mating in species where males provide no material benefits to females. It is also pertinent that we assess the importance of forced copulation in fruit flies given their extensive use in research on sexual conflict, sexual selection and incipient speciation.

To examine the issue of teneral matings in fruit flies (*D. melanogaster*), we conducted a series of experiments with three overarching goals. First, we wished to establish the prevalence, time window and general features of teneral matings. Second, we wanted to examine whether teneral matings are in fact forced. After establishing that teneral matings were rather frequent and constituted forced copulations, we evaluated the subsequent reproductive decisions of tenerally mated females. Specifically, we tested whether tenerally mated females attempted to override the outcome of male coercion by remating with a male of their choice once they reached sexual maturity, and whether teneral females' resistance to forced copulation was positively associated with their tendency to remate when mature.

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GENERAL METHODS

We used Canton-S *D. melanogaster* in all the experiments except for the study involving wild-caught flies (see below). The flies were kept in $20 \times 20 \times 35$ cm population cages containing a few thousand individuals inside an environmental chamber at 25 °C and 60% relative humidity, on a 12:12 h light:dark cycle. Each population cage had two standard 240 ml food bottles each containing 50 ml of standard fly medium made of water, corn meal, glucose, yeast, sucrose, agar and methyl paraben. The flies used in the experiment were developed at a low density in food bottles each containing about 200 larvae. We collected teneral females with no anaesthesia soon after eclosion. We collected all other flies using the standard method of anaesthetizing them with CO_2 within 8 h of eclosion and placing them in groups of 20 in single-sex food vials. We transferred the virgin 3-day-old males singly into vials and used them when they were 4 days old. We used each male only once in the tests. All data recordings were performed by observers unaware of our predictions, and, when feasible, blind to fly treatment. We used logistic regression to analyse the mating frequency data, and ANOVAs on arcsine square-root proportions of time and log-transformed time data. The transformed data met ANOVA's assumptions. We used planned contrasts in the experiments involving three treatments.

FREQUENCY AND TIME WINDOW OF TENERAL MATINGS

We started by conducting two preliminary experiments to examine whether teneral matings occur in our laboratory population, and, if so, whether they take place only during a limited time window following female eclosion. Furthermore, we also tested whether teneral matings occur in wild-caught local flies.

Methods

To test for teneral matings, we collected without anaesthesia 70 recently eclosed females and placed each in a vial with two mature males. As a control, we placed 70 4-day-old females each with two mature males. The test trials lasted 1 h and, where mating occurred, we recorded the mating latency and duration. Mating was defined as a continuous mounting lasting over 2 min, mating latency was the time elapsed from placing the females with males inside vials until mating commenced, and the mating duration was measured from the initiation of continuous mounting until the males and females separated.

To examine the time window of teneral matings, we compared the mating rates of females collected just after eclosion (age 0 min), and 30 min and 60 min posteclosion. Because courtship and mating may be influenced by the time of eclosion, and are affected by the time of day (Hardeland 1972), we conducted two comparisons, one with equal eclosion times among the three treatments and the other with a simultaneous test time. That is, in the same eclosion time condition, we collected females simultaneously and tested them either immediately, 30 min or 60 min later. In the same test time condition, we collected females 0, 30 and 60 min prior to the test and tested them simultaneously. All test trials lasted 1 h. We tested 120 females of each age treatment, half under the same eclosion time condition and half under the same test time condition.

To assess the prevalence of teneral matings in local wild *D. melanogaster*, we collected a few hundred flies at several sites in southern Ontario and placed them in a population cage inside an environment chamber. The flies laid eggs in regular food bottles as described above, and we used the eclosing adults in the experiment. We followed the protocol described above for the Canton-S
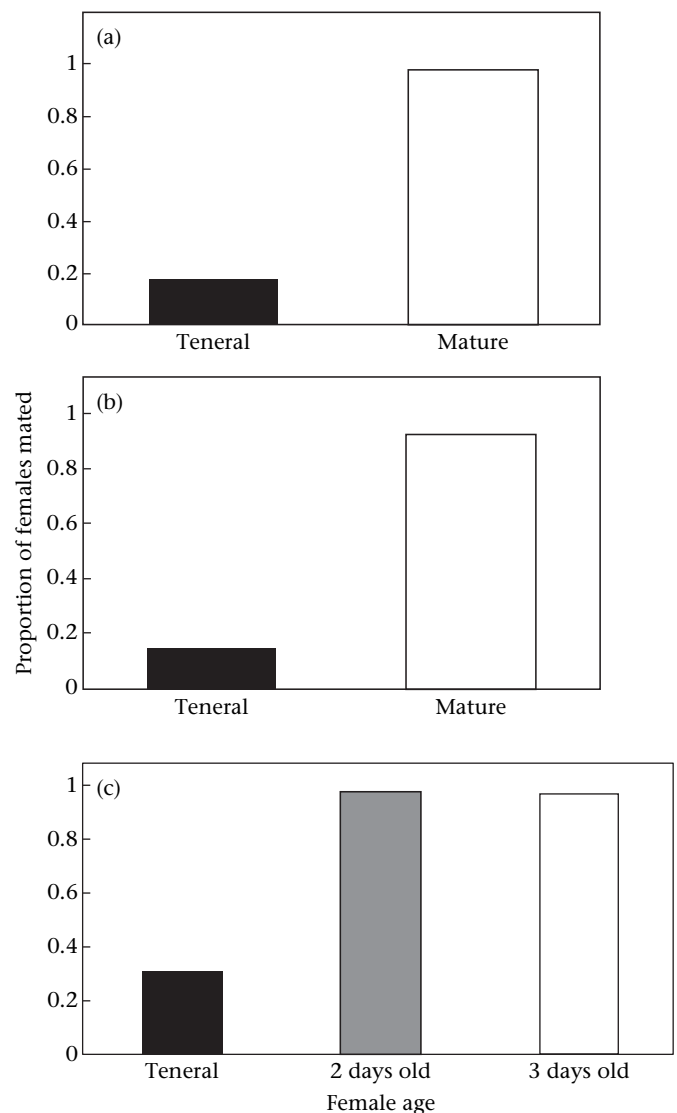

Figure 1. Proportion of matings by teneral and mature females in (a) the preliminary experiment with Canton-S flies ($N = 70$ females per group), (b) in the test with wild-caught flies ($N = 70$ females per group) and (c) during collection of mated females ($N = 657$ teneral females, 159 2-day-old females and 166 3-day-old females) for the remating test.

flies, using 90 teneral females and 90 4-day-old virgin females and a maximum of 2 h trial durations.

Results

In the test for teneral matings, 20% of the teneral females and close to 100% of the mature females mated within 1 h (Wald test: $\chi^2_1 = 41.1$, $P = 0.007$; Fig. 1a). The teneral females had much longer mating latencies ($F_{1,49} = 175.4$, $P < 0.01$; Fig. 2a) and shorter mating durations ($F_{1,49} = 12.0$, $P < 0.01$; Fig. 3a) than the mature, virgin females.

In the test for the time window of teneral matings, all but two matings occurred in the age 0 group. The frequency of matings was highest in the 0 age group in both the simultaneous eclosion time and simultaneous test time conditions (logistic regression: Wald test: $\chi^2_2 = 7.5$, $P < 0.05$ and $\chi^2_2 = 11.9$, $P < 0.005$, respectively; Fig. 4).

In the test using offspring of wild-caught flies, about 15% of the teneral females and 92% of the mature females mated (Wald test: $\chi^2_1 = 73.9$, $P < 0.001$; Fig. 1b). The teneral females had a longer mating
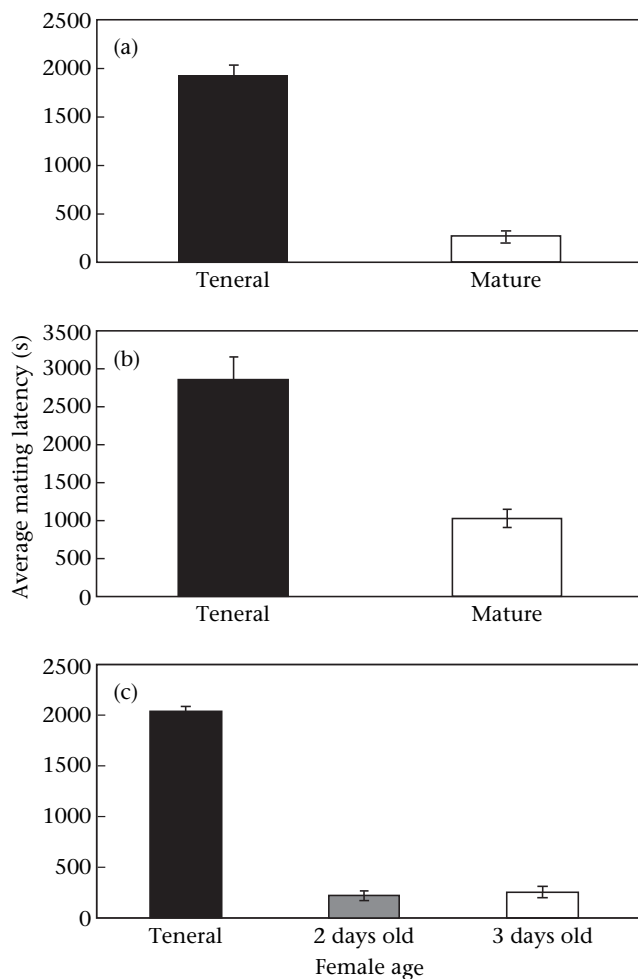
Figure 2. Average ± SE mating latency of (a) teneral ($N = 13$) and mature ($N = 38$) female Canton-S flies, (b) teneral ($N = 13$) and virgin mature ($N = 83$) females from a wild-caught population and (c) teneral ($N = 141$), 2-day-old ($N = 147$) and 3-day-old ($N = 148$) mature virgin female Canton-S flies during collection of mated females for the remating test.

latency ($F_{1,94} = 32.3$, $P < 0.01$; Fig. 2b) and shorter mating duration ($F_{1,94} = 12.9$, $P = 0.01$; Fig. 3b) than the mature virgin females.

OFFSPRING PRODUCTION FROM TENERAL MATINGS

Teneral matings are relevant only if tenerally mated females produce viable offspring. We examined this issue by quantifying the relative rate of adult progeny derived from teneral matings.

Methods

We used the same female treatments as in the remating experiment described below. That is, females mated as teneral or as sexually mature virgins, at either 2 or 3 days old. Each day after mating, we transferred females individually into fresh food vials. From day 14 to day 21, we transferred the females every other day as the rate of egg laying declined. We kept the vials for 2 weeks after egg laying and then anaesthetized and counted all the eclosed adult offspring. No further adults eclosed after that period. A preliminary analysis indicated a similar pattern of progeny production rate in the two mature female groups (repeated measures ANOVA: $F_{1,27} = 1.3$, $P = 0.26$), so we combined the two mature female groups.
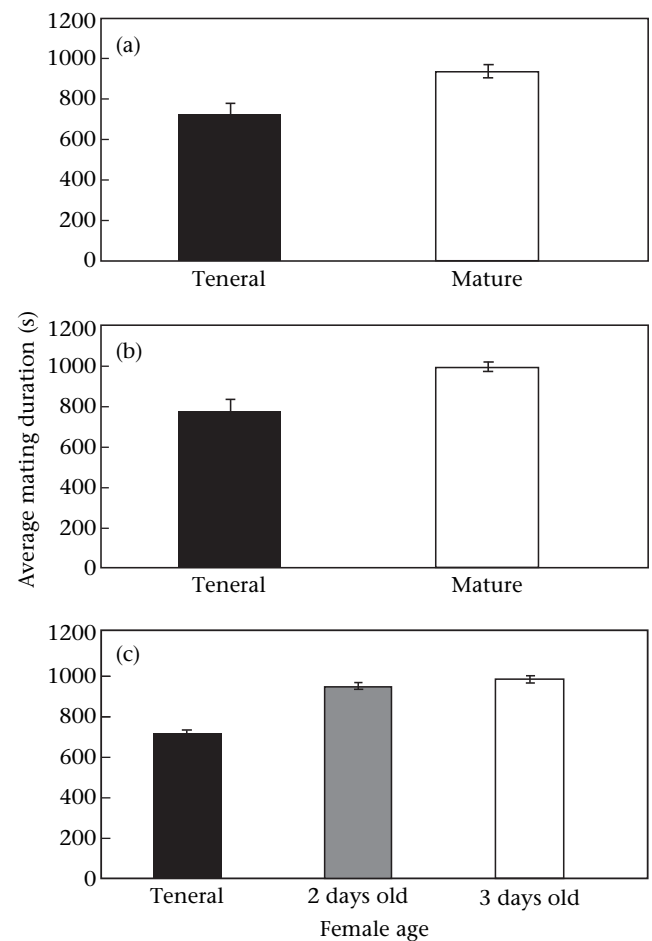

Figure 3. (a) Average ± SE mating duration of (a) teneral and mature female Canton-S flies, (b) teneral and virgin mature female wild-caught flies and (c) teneral, 2-day-old and 3-day-old mature virgin female Canton-S flies during collection of mated females for the remating test. Sample sizes are given in the legend of Fig. 2.

Results

The tenerally mated females produced about one-third as many adult progeny as the females mated when mature (repeated measures ANOVA: $F_{1,38} = 31.1$, $P < 0.001$), with much of the
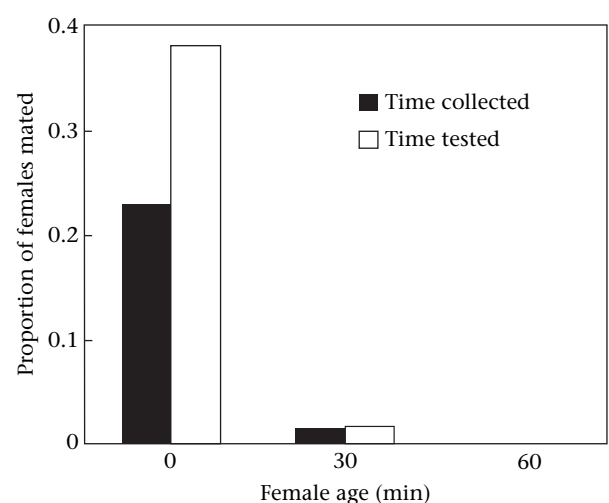

Figure 4. Proportion of teneral females age 0, 30 and 60 min that mated within 1 h. Females ($N = 360$) were either collected (■) or tested (□) simultaneously.
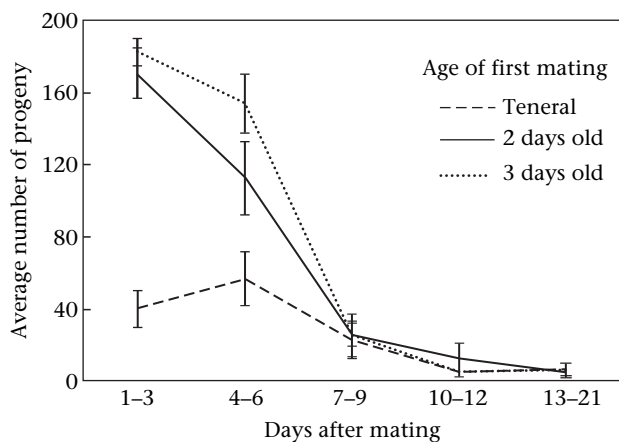
Figure 5. Average ± SE number of progeny produced by females that were teneral ($N = 11$), 2 days old ($N = 12$) or 3 days old ($N = 17$) when mated.

difference observed in the first 6 days following mating (Fig. 5). The range of total progeny was 5–303 for the teneral females and 99–563 for the mature females.

ARE TENERAL MATINGS FORCED? MALES' BEHAVIOUR AND FEMALES' RESPONSE

We expected the sexually immature teneral females to reject males' courtship attempts. Hence, either successful matings could indicate teneral females' tactic of avoiding further male harassment, or they could constitute forced copulations (Thornhill & Alcock 1983; Arnqvist & Rowe 2005). The results revealed a short time window of teneral matings (Fig. 4), which suggested that males succeeded in forcing copulations on only very recently eclosed females. We wished to quantify whether the males show obvious coercion and whether the teneral females attempt to

Figure 6. Male behaviour towards teneral and older sexually immature females during courtship: (a) grabbing; (b) mounting attempts. $N = 20$.

prevent copulations and try to abort them once they have commenced.

Methods

We conducted two separate comparisons focusing on fly behaviour during courtship and mating.

Courtship

The courtship observations involved 10 teneral females collected immediately after eclosion and 10 sexually immature females 2–10 h posteclosion. The sexually immature females are known to be able to deflect males' mating attempts (e.g. Manning 1967; Dukas 2010). We placed each female with a single male inside a small glass vial 10 mm in diameter and 5 mm high. We put the vial under a microscope at a $10\times$ magnification and video recorded the flies' behaviour for 15 min. We later scored the videos using the Observer software (Noldus Information Technology, Wageningen, The Netherlands).

For each of the 20 trials, we recorded the proportion of time that males spent courting, and their frequencies of grabbing females and of mounting attempts. Courtship included close following of the females and singing, grabbing involved the males using their front legs to hold the females, and mounting attempts consisted of the males moving into copulation positions that were aborted within a few seconds. For the females, we recorded the time they spent kicking the males with their hindlegs and their rate of movement, defined as the rate of crossing the midline of the small vial. Because the data involved multiple dependent variables, we used multivariate ANOVAs.

Mating

The mating observations involved 10 teneral females collected immediately posteclosion and 10 sexually mature, 4-day-old females. Here we focused on the females' behaviour and recorded the proportion of time they spent kicking the males (while either stationary or walking) and the proportion of time they spent walking without kicking. Typical mature female behaviour during mating involves little walking and kicking (R. Dukas, unpublished data). Data recording was similar to that during courtship except that we terminated the trials when the matings ended.

Results

Courtship

The males behaved differently towards teneral and immature females (multivariate ANOVA: $F_{3,16} = 38.8, P < 0.001$). Although the males spent similar proportions of time courting teneral and older immature females (0.96 ± 0.01 and 0.96 ± 0.008; $F_{1,18} = 0.15$, $P = 0.7$), they grabbed ($F_{1,18} = 121, P < 0.001$; Fig. 6a) and attempted to mount teneral females more than they did older immature females ($F_{1,18} = 6.8, P < 0.05$; Fig. 6b).

The teneral females behaved differently than the immature females (multivariate ANOVA: $F_{2,17} = 10.5, P < 0.001$). The teneral females moved slower ($F_{1,18} = 19.5, P < 0.001$; Fig. 7a) and spent a larger proportion of time kicking than the immature females ($F_{1,18} = 8.1, P < 0.02$; Fig. 7b).

Mating

The teneral females behaved differently than the mature females (multivariate ANOVA: $F_{2,17} = 20.2, P < 0.001$). The teneral females spent larger proportions of time walking ($F_{1,18} = 11$, $P < 0.005$; Fig. 7c) and kicking ($F_{1,18} = 14.1, P < 0.001$; Fig. 7d) than the mature females.
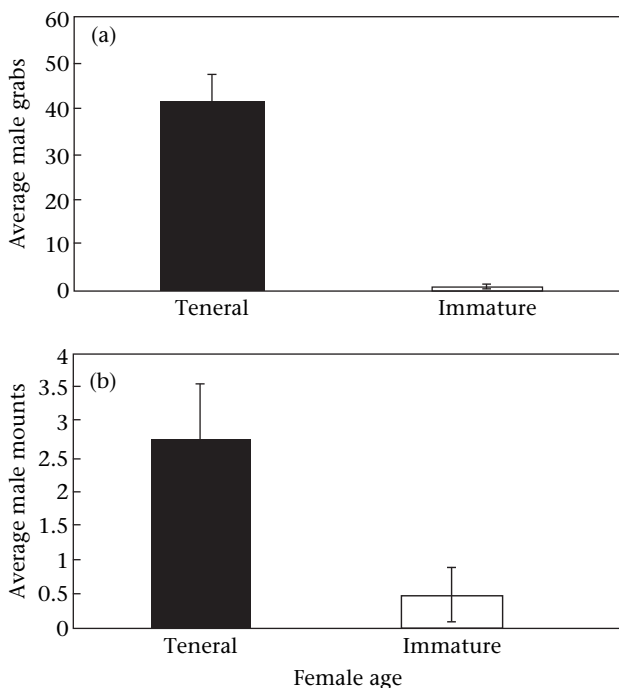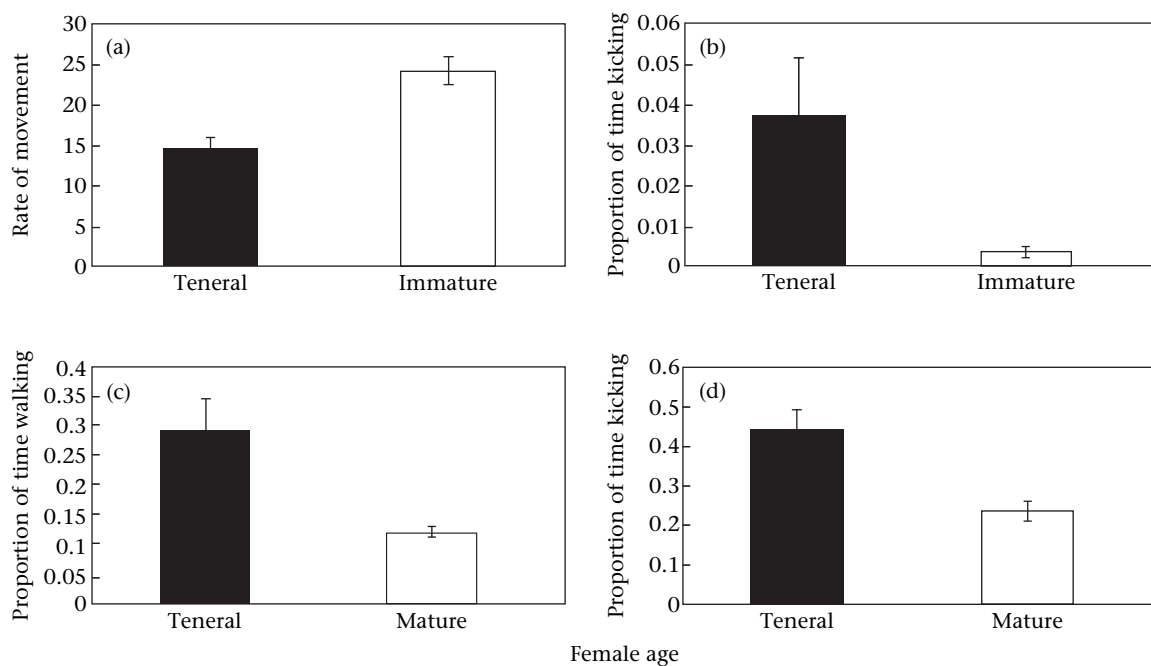
Figure 7. Average ± SE (a) rate of movement and (b) proportion of time spent kicking by teneral and older sexually immature females during courtship ($N = 20$ females), and the average ± SE proportion of time spent (c) walking and (d) kicking by teneral and sexually mature females during copulation ($N = 20$ females).

Figure 8. (a) Proportion of rematings of females that mated while teneral ($N = 141$), 3 days old ($N = 148$) and 2 days old ($N = 147$). The '3 and 6' group had a 3-day gap between mating and the remating test like the tenerally mated females, while the '2 and 3' group was tested at the same age as the tenerally mated females. (b) Average ± SE teneral mating duration of females ($N = 141$) that either remated or did not remate when sexually mature.

REMATING RATE OF TENERALLY MATED FEMALES

Our behavioural observations suggested that the males use force to attempt mating with teneral females and that the females keep fighting off the males even during copulation. By resisting the males during mating, the females could perhaps reduce the mating duration and hence receive less sperm and seminal fluid. Because both sperm and accessory proteins in the seminal fluid influence females' propensity to remate (e.g. Gromko et al. 1984; Ram & Wolfner 2009), females' resistance could increase their propensity to remate with a male of their choice once they reach sexual maturity. In fruit flies, as well as in many other insects, the last male to mate fathers most progeny (e.g. Gromko et al. 1984; Price et al. 1999; Snook & Hosken 2004). We tested the possibility of a higher remating propensity of tenerally mated females by comparing their remating frequency to that of two control groups of mature females. Furthermore, we tested whether the remating propensity among teneral females would be negatively associated with the duration of the first mating. Finally, because the data set included rich information about the first matings, we report that information as well.

Methods

Both the interval between the last mating and age can affect females' propensity to remate (Gromko et al. 1984). Hence, in addition to the tenerally mated females, our experiment included two groups of females mated after reaching sexual maturity. One control group was tested at the same age as the tenerally mated females while the other control group had the same interval between the first mating and the remating test. Specifically, the teneral females mated when 0 days old and were tested when 3 days old. One control group mated when 2 days old and was tested when 3 days old. The other control group mated when 3 days old and was tested when 6 days old. Tests of the 3 groups were conducted simultaneously. Both the trials for collecting mated females and the remating tests lasted for a maximum of 2 h.
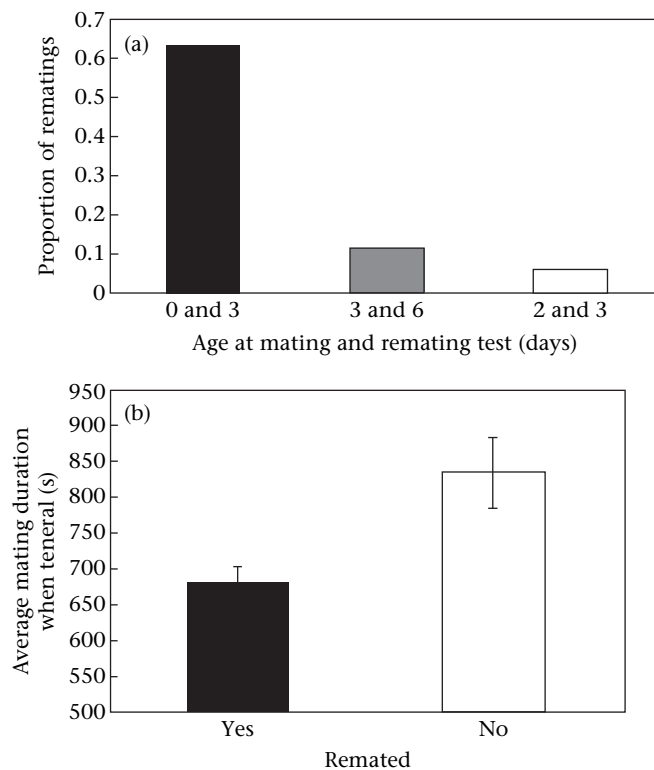
Results

First matings

About 30% of the teneral females and close to 100% of the mature 2- and 3-day-old females mated during the first mating trials (logistic regression: Wald test: teneral versus 2-day-old females: $\chi_1^2 = 78.4$, $P < 0.001$; teneral versus 3-day-old females: $\chi_1^2 = 89.2$, $P < 0.001$; Fig. 1c). The teneral females had longer mating latencies ($F_{2,433} = 457.4$, $P < 0.001$; Fig. 2c) and shorter mating durations ($F_{2,433} = 87.5$, $P < 0.001$; Fig. 3c) than the mature females.

Remating test

Tenerally mated females were much more likely to remate than females that mated when either 2 or 3 days old (logistic regression: Wald test: $\chi_1^2 = 73$, $P < 0.001$ and $\chi_1^2 = 70.1$, $P < 0.001$, respectively; Fig. 8a). The latencies ($F_{1,113} = 1.17$, $P = 0.3$) and durations ($F_{1,113} = 1.7$, $P = 0.18$) of rematings, however, were similar across the three treatments. Finally, the tenerally mated females that remated had a shorter teneral mating duration than those that did not remate ($F_{1,140} = 10.42$, $P = 0.002$; Fig. 8b).

DISCUSSION

Our results substantiate Markow's (2000) observations and indicate that teneral matings are a frequent occurrence in fruit flies (*D. melanogaster*) (Figs 1, 4). While we conducted our study under controlled laboratory settings, Markow (2000) observed teneral matings in local fruit flies (*D. melanogaster*) under seminatural settings at a citrus grove in Arizona, U.S.A. This indicates that our results are not merely a laboratory artefact. Furthermore, we used relatively large mating arenas (40 ml vials), two males per vial and short trials lasting only 1–2 h. While the confined space did not allow females to escape, teneral females cannot readily escape even in the wild because they cannot fly. In short, our results cannot be attributed to unrealistic settings.

Our results may help explain why no replication of Markow (2000) has occurred in the past decade. In an early attempt to replicate Markow's results, one of us (R.D.) failed to document teneral matings in females collected within 1 h posteclosion. In the current study, we found that almost all teneral matings occurred in females just after eclosion. Only 5% of the females collected 30 min after eclosion and no female 1 h posteclosion mated (Fig. 4). Intriguingly, a major distinction between Markow's (2000) data and ours is that Markow noted little or no courtship by the males and no resistance by the females, whereas we documented intense courtship by the males and strong resistance by the females before and during copulation (Figs 6, 7). We observed similar behaviours in flies of the wild-caught population. Perhaps there is between-population variation in the nature of male and female tactics as they relate to forced matings.

Are the teneral matings forced, or do they merely reflect male persistence and either female coyness or convenience mating? Although it is often difficult to distinguish between these alternatives (Thornhill 1980; Thornhill & Alcock 1983; Eberhard 1996; Arnqvist & Rowe 2005), a few lines of evidence indicate that teneral matings in fruit flies constitute forced copulations. First, substantial data indicate that female *D. melanogaster* do not typically accept males for the first several hours posteclosion (Manning 1967). Second, most of the teneral matings occurred in females just posteclosion while none took place in females 1 h after eclosion (Fig. 4). Furthermore, we have regularly used females 2–20 h old from the same population for courtship trials in which no matings occurred (Dukas 2010). Third, all three indicators of females' willingness to mate implied low mating propensity by the teneral females. The teneral females had lower mating frequencies (Fig. 1),

longer mating latencies (Fig. 2) and shorter mating durations (Fig. 3) than mature females. Fourth, our behavioural observations indicated that the recently eclosed, teneral females were more vulnerable than older sexually immature females and that males took advantage of that weakness. The teneral females were less agile and moved slower than older sexually immature females (Fig. 7a), so the males could readily grab them in preparation for mounting. Both grabbings and mountings occurred more often with teneral females than with older sexually immature females (Fig. 6). The teneral females fought off the male aggressive manoeuvres by kicking (Fig. 7b) and kept fighting during copulation (Fig. 7d). The teneral females' continuous resistance before and during copulations could explain the lower frequencies, longer latencies and shorter durations of teneral matings relative to those of mature matings (Figs 1–3).

The teneral matings produced progeny, although their numbers were much smaller than those from mature matings (Fig. 5). The likely reason for this difference is that the teneral females are not physiologically prepared for mating and the reproductive changes it triggers (Moshitzky et al. 1996). We do not have sufficient data to evaluate the fitness benefits to males nor the costs to females resulting from teneral matings. It is possible, however, that at least the males that are deemed unattractive by mature females can gain fitness from teneral matings. As for the females, in addition to having some proportion of their offspring fathered by a male they did not choose, they may incur another cost, not quantified here, of injuries that reduce survival, mating success and reproductive output. Injuries caused by forced copulation have been documented in a few species (McKinney et al. 1983; Thornhill & Palmer 2000; Arnqvist & Rowe 2005).

The teneral females could deflect many but not all males' persistent mating attempts (Fig. 1). Once copulation commenced, however, the teneral females still had two related means of resistance. First, the females kept obstructing the copulating males through walking and kicking (Fig. 7c, d). This behaviour may have caused teneral matings to be significantly shorter than mature matings (Fig. 3). The mating duration in *D. melanogaster* is assumed to be mostly under males' control (Krebs 1991; Jagadeeshan & Singh 2006), and our observations do not indicate that the teneral females could physically terminate matings. It is possible that the males responded to the teneral females' resistance by terminating the matings early. In the bean weevil (*Callosobruchus maculatus*), controlled experiments indicated that female kicking reduced mating durations (Crudgington & Siva-Jothy 2000). Females could also diminish the effects of teneral matings by exercising mate choice in subsequent rematings. Indeed, the tenerally mated females were over six times more likely to remate than control females (Fig. 8a). Furthermore, females that remated had shorter teneral matings than females that did not remate (Fig. 8b). Hence, if resistance during teneral mating is negatively associated with the duration of that mating, then such resistance would increase the likelihood of subsequent remating.

How significant are teneral matings for the ecology and evolution of fruit flies (*D. melanogaster*)? The fact that teneral matings occur in the wild (Markow 2000) and in our population of wild-caught flies (Fig. 1b) indicate that teneral matings cannot be ignored. While females can diminish the effects of teneral matings through subsequent rematings (Fig. 8a), teneral matings probably produce a non-negligible proportion of the females' lifetime offspring. Hence, teneral matings can decrease the rate of evolution via female mate choice. Furthermore, if males from a partially divergent population are rejected by mature females of another population and resort to forced, teneral matings, they can counteract the process of population divergence via female choice.

In summary, we documented about 20% matings in teneral female fruit flies. These matings were the outcome of male coercion countered by female struggle, which was only partially successful. The tenerally mated females, however, could reduce the number of offspring fathered via forced copulation by subsequent remating.

Acknowledgments

References

Arnqvist, G. & Rowe, L. 2005. *Sexual Conflict*. Princeton, New Jersey: Princeton University Press.

Clutton-Brock, T. H. & Parker, G. A. 1995. Sexual coercion in animal societies. *Animal Behaviour*, **49**, 1345–1365.

Coyne, J. A. & Orr, H. A. 2004. *Speciation*. Sunderland, Massachusetts: Sinauer.

Crudgington, H. S. & Siva-Jothy, M. T. 2000. Genital damage, kicking and early death. *Nature*, **407**, 855–856.

Dukas, R. 2010. Causes and consequences of male–male courtship in fruit flies. *Animal Behaviour*, **80**, 913–919.

Eberhard, W. G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton, New Jersey: Princeton University Press.

Fricke, C., Bretman, A. & Chapman, T. 2010. Sexual conflict. In: *Evolutionary Behavioral Ecology* (Ed. by D. F. Westneat & C. W. Fox), pp. 400–415. Oxford: Oxford University Press.

Gromko, M. H., Gilbert, D. G. & Richmond, R. C. 1984. Sperm transfer and use in the multiple mating system of *Drosophila*. In: *Sperm Competition and the Evolution of Animal Mating Systems* (Ed. by R. L. Smith), pp. 371–425. Orlando, Florida: Academic Press.

Hardeland, R. 1972. Species differences in the diurnal rhythmicity of courtship behaviour within the *melanogaster* group of the genus *Drosophila*. *Animal Behaviour*, **20**, 170–174.

Jagadeeshan, S. & Singh, R. S. 2006. A time-sequence functional analysis of mating behaviour and genital coupling in *Drosophila*: role of cryptic female choice and

male sex-drive in the evolution of male genitalia. *Journal of Evolutionary Biology*, **19**, 1058–1070.

Krebs, R. A. 1991. Function and genetics of long versus short copulations in the cactophilic fruit fly, *Drosophila mojavensis* (Diptera: Drosophilidae). *Journal of Insect Behavior*, **4**, 221–233.

McKinney, F., Derrickson, S. R. & Mineau, P. 1983. Forced copulation in waterfowl. *Behaviour*, **86**, 250–294.

Mallet, J. 2006. What does *Drosophila* genetics tell us about speciation? *Trends in Ecology & Evolution*, **21**, 386–393.

Manning, A. 1967. The control of sexual receptivity in female *Drosophila*. *Animal Behaviour*, **15**, 239–250.

Markow, T. A. 2000. Forced matings in natural populations of *Drosophila*. *American Naturalist*, **156**, 100–103.

Moshitzky, P., Fleischmann, I., Chaimov, N., Saudan, P., Klauser, S., Kubli, E. & Applebaum, S. W. 1996. Sex-peptide activates juvenile hormone biosynthesis in the *Drosophila melanogaster* corpus allatum. *Archives of Insect Biochemistry and Physiology*, **32**, 363–374.

Noor, M. A. F. & Feder, J. L. 2006. Speciation genetics: evolving approaches. *Nature Reviews Genetics*, **7**, 851–861.

Pitnick, S. & Hosken, D. J. 2010. Postcopulatory sexual selection. In: *Evolutionary Behavioral Ecology* (Ed. by D. F. Westneat & C. W. Fox), pp. 379–399. Oxford: Oxford University Press.

Price, C. S. C., Dyer, K. A. & Coyne, J. A. 1999. Sperm competition between *Drosophila* males involves both displacement and incapacitation. *Nature*, **400**, 449–452.

Ram, K. R. & Wolfner, M. F. 2009. A network of interactions among seminal proteins underlies the long-term postmating response in *Drosophila*. *Proceedings of the National Academy of Sciences, U.S.A.*, **106**, 15384–15389.

Ritchie, M. 2007. Sexual selection and speciation. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 79–102.

Rundle, H. D. & Boughman, J. W. 2010. Behavioral ecology and speciation. In: *Evolutionary Behavioral Ecology* (Ed. by D. F. Westneat & C. W. Fox), pp. 471–487. Oxford: Oxford University Press.

Snook, R. R. & Hosken, D. J. 2004. Sperm death and dumping in *Drosophila*. *Nature*, **428**, 939–941.

Spieth, H. T. & Ringo, J. M. 1983. Mating behavior and sexual isolation in *Drosophila*. In: *The Genetics and Biology of* Drosophila (Ed. by M. Ashburner, H. L. Carson & J. N. Thompson), pp. 345–384. London: Academic Press.

Thornhill, R. 1980. Rape in *Panorpa* scorpionflies and a general rape hypothesis. *Animal Behaviour*, **28**, 52–59.

Thornhill, R. 1984. Alternative hypotheses for traits believed to have evolved by sperm competition. In: *Sperm Competition and the Evolution of Animal Mating Systems* (Ed. by R. L. Smith), pp. 151–178. Orlando, Florida: Academic Press.

Thornhill, R. & Alcock, J. 1983. *The Evolution of Insect Mating Systems*. Cambridge, Massachusetts: Harvard University Press.

Thornhill, R. & Palmer, C. 2000. *A Natural History of Rape: Biological Bases of Sexual Coercion*. Cambridge, Massachusetts: MIT Press.