

Sexual conflict over copula timing: a mathematical model and a test in the yellow dung fly

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Sexual conflict over mating occurrence, timing, or duration is common in animals. This explains conspicuous female mate rejection behavior in many species, often involving shaking, fighting, and occasional forced copulations. We present a simple model that generates predictions about whether and when copulation occurs in such conflict situations and how much female rejection behavior should be observed. Predictions depend on 2 underlying parameters affecting female resistance and male persistence. We supply 2 qualitative tests of the model using the yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae). We manipulated adult age, body size (large and small), and adult food availability (low and high), independently in males and females, staging replicate pairings of all treatment combinations. In agreement with predictions of our model, shaking duration first increased to a maximum at intermediate age, when the average female copulated, and then decreased again. Contrary to expectation, body size did not affect copulation timing, female resistance, or male persistence. As predicted, adult food limitation delayed sexual maturity and hence prolonged female resistance, resulting in later copulations after more shaking. However, although food limitation equally delayed the increase in male persistence with age, copulation also occurred later after more shaking, opposite to the model prediction. We conclude that shaking is driven primarily by female age and male responses to it. Although female shaking can initially successfully deter males in *S. stercoraria*, this behavior is subtle and has apparently shifted function from an effective means of mate choice to a signal of nonreceptivity, though its importance in nature remains unclear. *Key words*: body size, copulation duration, food limitation, *Scathophaga stercoraria*, *Scatophaga*, sexual conflict, sexual selection. [*Behav Ecol* 18:958–966 (2007)]

Mating has an obvious element of mutual benefit for both mating partners, but it is also loaded with conflict (Parker 1979; Thornhill and Alcock 1983; Eberhard 1996; Holland and Rice 1998; Chapman et al. 2003; Arnqvist and Rowe 2005). Although males should generally maximize their number of matings, females typically need only few copulations to assure fertilization of all their eggs (Darwin 1871; Bateman 1948). Optimal copulation frequency depends on a variety of associated fitness costs and benefits and will usually be lower for females than males (Arnqvist and Nilsson 2000). When a male and a female meet to reproduce, there is thus sexual conflict over whether and when copulation occurs. This explains conspicuous female mate rejection behavior in many species, often involving shaking, fighting, occasional forced copulations, and even sexual cannibalism (Thornhill and Alcock 1983; Clutton-Brock and Parker 1995; Gowaty and Buschhaus 1998; but see Eberhard 2002; e.g., Rowe et al. 1994; Blanckenhorn et al. 2000; Elgar et al. 2000). Who “wins” this conflict ultimately depends on who has greater behavioral control over the outcome of mating, which depends to some degree on which sex is larger (Ding and Blanckenhorn 2002). Here we develop a simple model that generates predictions about whether and when copulation occurs in such conflict situations and how much female rejection behavior may be observed. We test the model with 2 experiments on yellow dung flies, *Scathophaga stercoraria* (L.) (sometimes *Scatophaga*; Diptera: Scathophagidae), a classic model species for studies

of sexual selection and conflict (Parker 1970a, 1979; Simmons 2001).

As mating is per definition an interaction between the sexual partners, its outcome depends on both female and male behavior (Arnqvist and Rowe 2005). In accordance with the fundamental differences between the sexes outlined above, we call these behaviors female resistance to mating and male persistence in mating. Both should be regarded as unmeasured, underlying state variables that depend on a variety of extrinsic (e.g., nutrition, temperature, etc.) and intrinsic (e.g., body size, the reproductive cycle, etc.) parameters and the costs and benefits of which are dynamic and can shift over time (e.g., Ortigosa and Rowe 2002). The result of this interaction to be predicted is the duration of (female) shaking or struggling and whether and when copulation occurs.

It is important to note that female resistance and male persistence could reasonably be and have in the past been estimated by the amount of (female) shaking and the number of male mating attempts per unit time, respectively (e.g., Arnqvist 1992; Rowe 1992; Rowe et al. 1994; Crean and Gilburn 1998; Blanckenhorn et al. 2000; Ortigosa and Rowe 2002). However, these estimates and the presumed underlying variables are not quite the same, as the measured behavior results from an interaction of the underlying variables (persistence and resistance), which may not be directly measurable. This becomes intuitively clear when considering the extreme outcomes of a continuum. If females are willing to copulate with any male, copulation will result after only one mating attempt, and therefore, the number of mating attempts cannot be a good estimate or predictor of male persistence in this situation. Similarly, cumulative shaking duration can be prolonged if males keep trying but may be very short if males give up early, potentially yielding 2 very different estimates of the reluctance of a particular female to mate.

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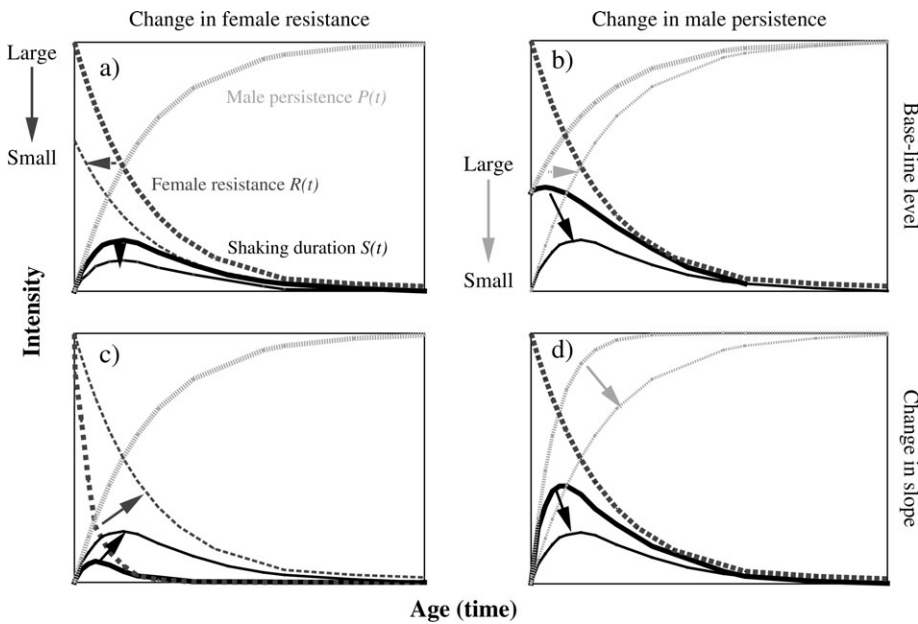


Figure 1
 Qualitative change in the functions of female resistance (dark gray, decreasing hatched lines), $R(t)$, male persistence (light gray, increasing dotted lines), $P(t)$ (both exponential here), and the resulting predicted shaking duration, $S(t)$ (black, hump-shaped solid lines), when (a) the baseline resistance decreases as female body size decreases, (b) the baseline persistence decreases as male body size decreases, (c) female resistance decreases more slowly as a function of food restriction, and (d) male persistence increases more slowly as a function of food restriction. In all cases, copulation is predicted to occur when $S(t)$ reaches a maximum.

We investigate the dependence of shaking behavior and copulation occurrence on time t or age ($t > 0$), which are identical and interchangeable here and can be treated independently for the sexes so that males and females do not have to be of the same age. From first principles, we assume that a female’s resistance to mate, $R(t)$, best viewed as the probability that she resists a mating attempt, should decrease with time (age) in most situations: as virgins approach sexual maturity, because her residual reproductive value necessarily decreases with age, or because sperm reserves become depleted. For similar reasons, male persistence in mating, $P(t)$, that is, the probability of mating attempts given encounter, can be expected to increase with time as males mature or age. This is because both sexes should maximize their reproductive output. We thus envision probabilistic exponential functions that start at some initial, baseline maximum for $R(t)$ or minimum for $P(t)$ and decrease (increase) monotonically toward a minimum (maximum), which, however, is never reached because age always progresses, so a minimal level of resistance and increase in persistence always remains. We further assume that the 2 functions overlap over the entire valid parameter space and cross at some point $t > 0$. Thus, we can in principle model the whole lifetime of an individual, any particular episode of their reproductive life, or one single mating encounter. Figure 1 shows this graphically, and a formal treatment is given in the Appendix.

Two sets of parameters affect the position and shape of the curves in Figure 1. On the one hand, the initial levels of $R(t)$ and $P(t)$, that is, the Yintercept parameters c_F and c_M (see Appendix), can be viewed biologically as reflecting intrinsic (i.e., potentially genetic) variation in resistance or persistence among individuals. This may depend, for instance, on body size in that large-bodied females or males may be expected to show stronger resistance or persistence. In this sense, initial resistance or persistence will primarily reflect evolutionary adaptation due to past selection or (physiological) constraints. Intuitively, females are more likely to determine the outcome of mating in species with larger females, and some level of baseline resistance or shaking behavior is consequently likely to have evolved (Ding and Blanckenhorn 2002). In contrast, the baseline male persistence may be zero, for example, when males are sexually immature and cannot mate, but it may also be high right from the start. In species with larger males, one

might even expect male persistence to exceed female resistance (i.e., $P(t) > R(t)$) at all times, in which case (forced) copulation is predicted to occur with certainty after little to no resistance.

On the other hand, the slopes of the functions, that is, the parameters p and r , reflect the magnitude of change in resistance or persistence with time or age (Figure 1; cf., Appendix). Biologically, this can be viewed as being caused primarily by variation in extrinsic or environmental factors that have been shown or argued in the past to influence female resistance and male persistence in mating, most notably nutrition, temperature, or the precise time position in the reproductive cycle, which in turn depends on the environment (e.g., Rowe et al. 1994; Blay and Yuval 1997; Yuval et al. 1998; Blanckenhorn et al. 2000; Ortigosa and Rowe 2002; Teuschl and Blanckenhorn 2007). We here envision and briefly discuss 4 such potential effects, although additional scenarios are conceivable. 1) When organisms need to feed to attain sexual maturity in the first place, as is the case for anautogenous or income-breeding insects, they should initially be unreceptive to the opposite sex, so male persistence should initially be low (or nil) and female resistance high. This is because both sexes would waste time and energy by mating instead of foraging to mature eggs and sperm (Blanckenhorn et al. 1995), thus assuming some cost of premature matings. Feeding should then cumulatively promote sexual maturation and hence continuously diminish female resistance to mate and increase male mating persistence. This maturation process should be faster if food is more abundant. 2) On the other hand, given individuals are sexually mature, that is, if the reproductive machinery is in place, lack of nutrition (or hunger level) is likely to reduce the energy expendable in resistance and persistence. As food becomes less available, resistance $R(t)$ would then be expected to decrease more steeply and persistence $P(t)$ to increase less steeply (cf., Ortigosa and Rowe 2002). 3) Body size has been argued above to potentially affect intrinsic levels of resistance or persistence. Additionally, however, body size could also affect the time- or age-dependent change in resistance or persistence. For example, larger females might have larger sperm stores but are also likely to deplete their stored sperm faster because their fecundity is greater. This would imply higher initial resistance but a faster decline of resistance with time. An analogous argument can

Table 1

Predicted qualitative changes in the time of copulation (= time of maximal shaking), t^* , and the resulting shaking duration, S^* , as a function of a decrease in the initial resistance or persistence (model parameters c_F and c_M), here presumably effected by a change in body size, or a change in the slope of the resistance, $R(t)$, and persistence, $P(t)$, functions (model parameters r and p)

Biological effect	Model parameter	Time of copulation/ maximal shaking t^*	Shaking duration S^*
Initial resistance decrease (Figure 1a)	$c_F \uparrow$	0	\downarrow
Initial persistence decrease (Figure 1b)	$c_M \downarrow$	\uparrow	\downarrow
Slower decline of resistance with time (Figure 1c)	$r \downarrow$	\uparrow	\uparrow
Slower incline of persistence with time (Figure 1d)	$p \downarrow$	$\uparrow (\downarrow)$	\downarrow

Changes in the opposite direction are completely symmetrical.

be made for male persistence in relation to sperm reserves. 4) Instead of absolute age, the time position in the reproductive cycle might be the major factor driving female resistance, which consequently might be sinusoidal (Teuschl and Blanckenhorn 2007). Analogously, male persistence might depend on sperm reserves, which needs to be replenished periodically after use. In both cases the model should be applied to each cycle, starting after each oviposition or feeding bout.

Our model generally serves to predict the qualitative changes in the time of maximal shaking (t^*) and the corresponding amount (or intensity) of shaking (S^*) as a function of variation in the baseline levels of (c_F or c_M) and time-dependent changes in resistance or persistence (r or p). These general predictions are summarized in Table 1 and formally treated in the Appendix. t^* is also the time at which this deterministic model predicts that copulation should occur as a step function. This is intuitive because at this point the functions $R(t)$ and $P(t)$ cross (Figure 1) and male persistence becomes relatively stronger than female resistance. In a stochastic version of the model, as in any empirical test fraught with estimation error, the switch (from no copulation) to copulation at time t^* would actually be sigmoid in shape instead of being a step function.

We here describe results from 2 laboratory experiments applying the model to the particular scenario (1) described above. We first demonstrate the validity of the model with a simple experimental data set (Study 1). We then test the specific qualitative model predictions outlined in Figure 1 with a second experimental study (Study 2), treating the case when body size presumably affects the initial, baseline resistance and persistence (Figure 1a,b) and when food delays the attainment of sexual maturity in males and females (Figure 1c,d). We consequently manipulated age (t), body size, and adult food availability. We independently manipulated males and females, permitting differentiation of male and female effects because their predictions are qualitatively different (Figure 1; Table 1).

We tested the model with yellow dung flies (*S. stercoraria* L.). Precopulatory sexual selection in *S. stercoraria* is largely mediated by male–male competition (Parker 1970b, 1970c; Simmons 2001). Males of this species are larger than females (the uncommon case in insects), probably because sexual selection favoring large male size is consistently strong in nature

(Borgia 1981; Jann et al. 2000; Kraushaar and Blanckenhorn 2002; Blanckenhorn et al. 2003). Consequently, males can force copulations to some extent, and females appear to have few means to choose or avoid males at the dung, their oviposition site, other than by timing or directing their approach toward particular males (Parker 1970c; Borgia 1981; Reuter et al. 1998). Females therefore mate multiply and store sperm so that sperm competition is common (Parker 1970a, 1970b), and cryptic sperm choice by females has been reported (Ward 1998, 2000). Using optimality models, Parker (1970a, 1970b) and coworkers (summarized in Simmons, 2001, p. 198ff) concluded that copula duration in yellow dung flies depends on several factors but is largely determined by the male. However, both Parker (1970c) and Borgia (1981) described mated females regularly performing brief up-and-down and sometimes side-to-side “bucking” movements that typically last for only few seconds (but are often shorter than 1 s), much like push-ups but not unlike the much more prominent shaking behavior that other insects perform when trying to dislodge mounted males (e.g., Rowe et al. 1994; Blanckenhorn et al. 2000). This is the (shaking) behavior we focus on here. Females also similarly shake toward the end of copulation, presumably to encourage the male to dismount, although in many cases such female shaking appears to have little detectable effect on the male, who typically clings on.

MATERIALS AND METHODS

Study 1

In this study, which served primarily to validate the general qualitative model predictions, we manipulated female age (t) while randomizing and hence controlling for male age and any other effects across all female age classes. Consequently, the presumed but unmeasured male persistence function $P(t)$ was held constant, in that average male persistence was identical for all female age classes; any resulting effects would therefore be attributable to females only.

Sexually immature females aged 2–10 days past adult emergence were confronted with sexually experienced and mature males ready to copulate (randomized adult ages between 24 and 37 days). At optimal laboratory conditions, females take at least 10 days to mature their first clutch of eggs (Blanckenhorn and Henseler 2005). We observed the number of male mating attempts, the cumulative duration of female shaking to dislodge mounted males before copulation occurred, and whether and when copulation occurred.

All individuals used were offspring reared in the laboratory at 20 °C from parents collected at our field site in Fehraltorf, Switzerland. A range of male and female body sizes was generated by manipulating the amount of dung a set number of larvae had as food (Ding and Blanckenhorn 2002). Adult flies were held singly in 100-ml bottles and fed water, sugar, and ad libitum *Drosophila melanogaster* as prey. Yellow dung flies are nutritionally autogenous, requiring protein from prey to become sexually mature (Foster 1967), whereas energy for flight and other activities is primarily derived from sugar. Otherwise, standard rearing methods were used (see e.g., Ward and Simmons 1991; Ward 2000; Ding and Blanckenhorn 2002).

Each individual was only used once, although each male had copulated once before to assure sexual maturity. For all 5 female age categories tested (2, 4, 6, 8, and 10 days old), 2 replicates each of all 4 combinations of small and large mating partners were staged (Ding and Blanckenhorn 2002) to investigate the natural wide range of body sizes (estimated as hind tibia length: Parker and Simmons 1994). This yielded a total sample size of $N = 5 \times 2 \times 4 = 40$. Individuals were assigned

to the small and large size class by eye based on experience. Note that by adding a body size treatment, we additionally tested for presumed size effects as in Study 2. Otherwise mating partners were chosen at random, and the timing of the mating trials was also randomized with respect to age class and size. To mimic the natural situation, a female was added to a male waiting in a 100-ml bottle on a smear of dung, at which time observations began. Observations took place at room temperature (ca. 22 °C) and ended after 60 min or when copulation was terminated, whichever occurred first.

For each encounter, we expressed male persistence as the number of male mating attempts per minute and female reluctance as the cumulative amount of shaking that occurred until copulation ensued or the observation time was over. These 2 measures best reflect male persistence and female reluctance here because in our laboratory setting repeated encounters were possible (which do occur in nature, although admittedly females could ultimately not escape males in our vials). We analyzed the results using generalized linear models (GLM; in case of shaking duration and male mating attempts, which were square root transformed to better approximate a normal distribution of residuals) or binary logistic regression (in case of copulation occurrence), with female (i.e., experimental) and male (i.e., tester) body size class as fixed factors and female adult age as a continuous covariate (while not entering male age because it was randomized and we therefore did not expect any effects). We alternatively performed the analysis with both male and female hind tibia length entered instead as continuous covariates, yielding the same qualitative results and conclusions. We used SPSS 11 for MacIntosh for analysis.

Study 2

This study was similar to Study 1, except that there was an additional adult food treatment. Here we tested the presumed effects of food limitation on the decrease in female resistance and increase in male persistence with time, that is, the slopes of the functions $R(t)$ and $P(t)$, when attaining sexual maturity for the first time. In this case, food limitation should lead to a slower (i.e., shallower) decline in $R(t)$ (mediated by a decrease in parameter r ; Figure 1c) and a slower (i.e., shallower) incline in $P(t)$ (mediated by a decrease in parameter p ; Figure 1d; Table 1). In addition, we tested the effect of a decrease in body size, presumably leading to a decrease in the initial female resistance (i.e., a shift to the left of $R(t)$, mediated by an increase in a_f ; Figure 1a), and a decrease in the initial male persistence (i.e., a shift to the right of $P(t)$, mediated by a decrease in c_M , Figure 1a). As argued for Study 1, we did these manipulations independently for males and females, thus randomizing and hence controlling for the respective functions $P(t)$ and $R(t)$, particularly age effects of the other sex.

High food was ad libitum *D. melanogaster*, as in Study 1, whereas low food consisted of 10 *D. melanogaster* per week, administered twice or thrice a week, which has been shown to be limiting (Jann and Ward 1999). Both males and females received both food treatments but were then tested with well-fed, sexually experienced, mature partners that had previously copulated and/or laid eggs (adult age range 14–56 days). For the high-food individuals, there were again 5 age classes (2, 4, 6, 8, and 10 days old), whereas for low-food individuals age classes 12, 14, and 18 days were additionally tested because prey limitation delays sexual maturity (Blanckenhorn and Henseler 2005). There were again 2 size classes (small and large) of experimental males and females that were each tested with both size classes of tester partners, with 3 replicates of all 4 combinations of small and large mating partners. This

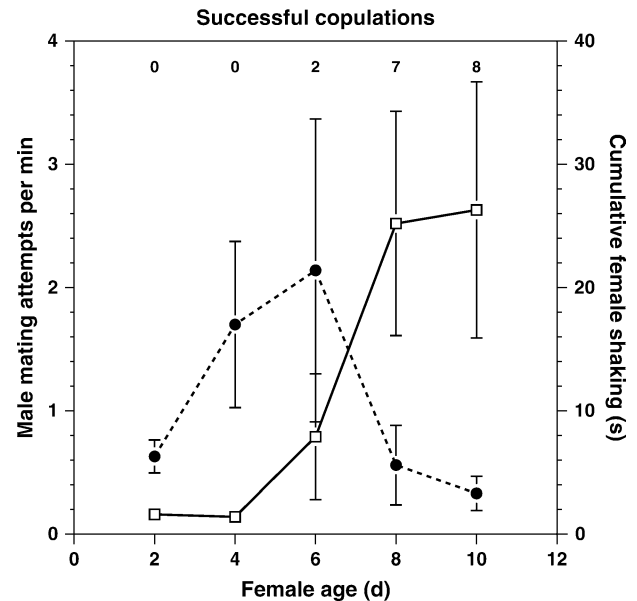


Figure 2

Mean \pm SE number of male mating attempts per minute (squares and solid line), cumulative female shaking (i.e., the predicted $S(t)$; circles and broken line), and number of successful copulations as a function of female age after emergence in Study 1 ($n = 8$ per age class).

yielded a total sample size of 5 age classes \times 4 size class combinations \times 2 food treatments \times 3 replicates plus the extra 3 age classes \times 4 size class combinations \times 3 replicates = 156 pairings per sex, although the sample size was somewhat diminished by missing values. In this experiment, we terminated observations at room temperature after 30 min or after copulation ensued, whichever occurred first.

We similarly analyzed the data using GLM (shaking duration and male attempts, both square root transformed) or logistic regression (copulation occurrence) with sex, food treatment, and experimental and tester body size class as fixed factors and female age and its square (to test for expected nonlinear effects; Figure 1) as continuous covariates (again deliberately omitting tester age because it was randomized). In the final model, we entered the experimental individuals' hind tibia length as continuous covariates instead of size class (with the same qualitative results and conclusions). We performed an analysis of both the female and male diet manipulations combined, which was possible and efficient because they were symmetrical; in this case, we specifically expected interactions because the model predicts shifts in opposite directions (Table 1; Figure 1). However, we additionally performed the simpler, separate analyses.

RESULTS

Study 1

The probability of copulating increased with female age in a sigmoid, threshold fashion, as expected and predicted by the model (Figure 2; logistic regression: = 8.36, $P = 0.004$). The number of male attempts per unit time, a reasonable standardized measure of male persistence, increased with female age ($F_{1,36} = 29.11$, $P < 0.001$; Figure 2). In contrast, the extent of female shaking first increased and then decreased with age, as predicted by the model (quadratic effect: $F_{2,36} = 3.44$, $P = 0.047$; Figure 2). Furthermore, for the age classes 6 and 8 days, the only age classes that exhibited variation in

whether copulation occurred or not (Figure 2), female shaking tended to be more pronounced when copulation did not ensue than when it did (mean \pm standard error [SE]: 23.4 ± 9.9 vs. 5.7 ± 8.7 s per attempt; $F_{1,14} = 3.43$, $P = 0.085$). The effects of male or female body size plus all interactions were never significant ($P > 0.2$), even though our size manipulation clearly created distinct size classes of substantial size difference (mean \pm SE hind tibia length of large males: 3.27 ± 0.026 mm; small males: 2.60 ± 0.034 mm; large females: 2.78 ± 0.020 mm; small females: 2.33 ± 0.028 mm).

Study 2

As in Study 1, our size classes were distinct and different (large males: 3.81 ± 0.037 mm; small males: 3.19 ± 0.034 mm; large females: 3.45 ± 0.023 mm; small females: 2.91 ± 0.029 mm). Body size of the tester individual had no effects whatsoever, nor did tester age (as χ^2_1 expected because they were randomized); both variables were consequently excluded from the final models.

As predicted (Figure 1c,d), the probability of copulating increased with female age (logistic regression: = 48.8, $P < 0.001$), and this increase occurred later when food was restricted (age by diet interaction: $\chi^2_1 = 36.6$, $P < 0.001$; all other effects, including the diet main effect and body size, were nonsignificant; Figure 3). Note, however, that contrary to Study 1 (Figure 2), 100% copulation was never reached in Study 2 (Figure 3). This probably occurred because not all mature females laid eggs at the time of testing, as would normally be the case when females show up at the dung in nature.

As assumed (Figure 1), male mating attempts generally increased with male or female age (age effect of experimental individuals), and this increase was steeper at high food (diet by age interaction; Table 2; Figure 4). The number of male attempts per minute was overall lower when male diet was manipulated than when female diet was manipulated (sex effect when analyzing both experiments together, Table 2; Figure 4). Contrary to expectation (Figure 1a,b), body size or its interactions with other factors had no effect on male mating attempts. The experimental age effect remained when the male and female diet manipulation experiments were analyzed separately, but the diet by age interaction was significant only in females (Table 2; Figure 4). These results demonstrate that the diet manipulation had the expected effect on male persistence.

The predicted hump-shaped effect of age on shaking duration generally occurred (Figure 1), as indicated by significant quadratic age effects in Table 2 (Figure 5). There was a further trend of a diet by age² interaction, indicating a shift in the age at which shaking was maximal (Table 2; Figure 5). Again, contrary to expectation (Figure 1a,b), body size (plus its interactions) had no effect on female shaking. Crucially, and again contrary to prediction (Table 1; Figure 1), diet manipulation of males and females had the same qualitative effect on age-dependent shaking duration, which interestingly was even stronger when male diet was manipulated (Figure 5b vs. 5a). When food was restricted, shaking overall increased (diet effect in Table 2; Figure 5). Finally, there was overall less female shaking when male diet was manipulated (sex effect in Table 2 when analyzing both experiments together; Figure 5a vs. 5b).

DISCUSSION

As many other insects (Thornhill and Alcock 1983), yellow dung fly females perform shaking behavior, presumably to resist unwanted matings. Unlike in other insects (e.g., Rowe et al. 1994; Blanckenhorn et al. 2000), however, this behavior is brief, subtle, and often of little apparent consequence, per-

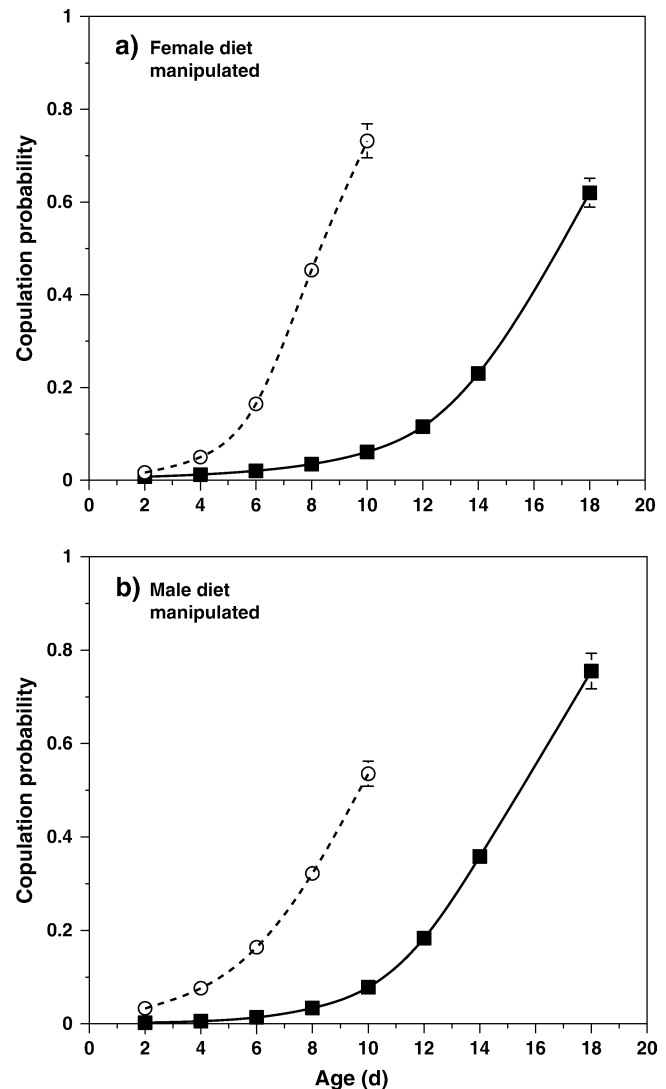


Figure 3 Realized copulation probability (i.e., proportion of resulting copulations) at high (open circles and dashed line) and low food (closed squares and solid line) when (a) female and (b) male diet was manipulated (Study 2). Nonparametric regression fits are indicated.

haps because yellow dung fly males are larger than females and thus can and do force copulations to some extent (Parker 1970c; Borgia 1981; Clutton-Brock and Parker 1995; Ding and Blanckenhorn 2002; but see Eberhard 2002). Nevertheless, immature females at least initially appear to successfully discourage males from copulating by shaking.

Both of our studies are at least qualitatively consistent with our model in that shaking duration generally first increased and then decreased again with age (Figures 2 and 5), as predicted (Figure 1). In Study 1, maximum shaking occurred at an intermediate age of about 6–8 days, when the average female copulated (Figure 2), marking the inflection point corresponding to the predicted step function shift from no copulation to copulation (Figure 1). Maximum shaking also occurred at the same time in Study 2 under high-food conditions (Figure 5). Our model predicts this outcome based on shifting costs and benefits of mating (Parker 1970c; Borgia 1981). This prediction was derived assuming 2 underlying variables, one in each sex, that are difficult or even impossible to measure directly: female resistance to mate, which can be

Table 2

Final analysis of variance models for the number of male mating attempts per minute (top) and the cumulative shaking duration (square root transformed; bottom) for both experiments combined as well for the female and male diet experiments separately

	Both				Females				Males		
	df	MS	F	P	df	MS	F	P	MS	F	P
Attempts											
Sex	1	1.476	13.092	<0.001							
Body size	1	0.164	1.458	0.228	1	0.098	0.794	0.374	0.090	0.845	0.360
Diet	1	0.078	0.689	0.407	1	0.102	0.829	0.364	0.003	0.027	0.869
Age	1	4.447	38.175	<0.001	1	2.101	17.026	<0.001	2.225	20.907	<0.001
Size × Diet					1	0.064	0.516	0.474	0.013	0.123	0.726
Size × Age	1	0.301	2.668	0.105	1	0.383	3.106	0.080	0.031	0.296	0.587
Diet × Age	1	0.740	6.568	0.011	1	0.852	6.904	0.010	0.072	0.673	0.413
Error	299	0.113			147	0.123			0.106		
Shaking											
Sex	21.998	12.375	0.001								
Body size	1	1.799	1.012	0.315	1	3.820	1.778	0.184	2.206	1.740	0.189
Diet	1	14.909	8.385	0.004	1	7.586	3.532	0.062	8.747	6.904	0.010
Age	1	8.065	4.536	0.034	1	15.662	7.291	0.008	2.520	1.988	0.161
Diet × Age					1	6.876	3.201	0.076	0.446	0.352	0.554
Age ²	1	21.276	11.966	0.001	1	18.674	8.693	0.004	3.784	2.987	0.086
Diet × Age ²	1	6.882	3.771	0.061	1	8.818	4.105	0.045	3.935	3.106	0.080
Error	299	1.778			147	2.148			1.267		

df, degrees of freedom; ms, mean square.

expected to diminish as females age and approach sexual maturity (in the laboratory at about 10 days after adult emergence: Blanckenhorn and Henseler 2005), and male persistence to mate, which can be expected to increase as males age and approach sexual maturity. Although female resistance will, indeed, generally be difficult to measure independent of male persistence, male persistence can be reasonably estimated in a standardized way by his number of mating attempts per unit time, as shown in Figures 2 and 4. This is because a male mating attempt is, at least at its inception, to a large degree independent of female behavior; this is not true for female shaking, which is entirely contingent on a male mating attempt. However, the number of male mating attempts ultimately also depends on the presence of female resistance because in the extreme of no resistance whatsoever, only one mating attempt will be necessary. Our approach makes explicit that the behavioral outcome and the underlying variables are not the same.

Although Study 1 served primarily to demonstrate the realism of our model, with Study 2 we specifically tested the predicted qualitative effects of variation in the baseline values (model parameters c_F and c_M ; discussed in the next paragraph) and the age-dependent change in resistance and persistence (parameters r and p ; Table 1). We addressed one of several concrete scenarios outlined in the Introduction, namely scenario 1, when food limitation delays attainment of sexual maturity in anautogamous insects (or any other organism), leading to a slower decline of female resistance (Figure 1c) and a slower incline of male persistence (Figure 1d). In both cases, the model predicts that copulation should occur later when food is restricted (Figure 1c,d), which is intuitive and what we found (Figure 3). Figure 4b further shows that food reduction produced the intended delay in male persistence, here estimated by his number of mating attempts per unit time. However, interestingly, the same delay in male persistence also resulted when female diet was manipulated (Figure 4a). This suggests that males respond to a female delay in sexual maturity, implying relatively higher resistance levels, by reducing their mating attempts. Such a response in male behavior to female behavior or reproductive state is

conceivable and implicit in our model (as discussed further below). Crucially, the model predicted an increase in shaking (S^*) when female diet was restricted (Figure 1c) but a decrease when male diet was restricted (Figure 1d), the former presumably mediated by relatively higher female resistance and the latter by relatively lower male persistence. However, this is not what we found: the results for the female and male manipulations are qualitatively identical (Figure 5). How can we interpret this result? On the one hand, the fact that the female but not the male age manipulation matched the model prediction may lead us to conclude that the female role is more important, the male merely responding to female behavior. On the other hand, the number of male mating attempts (i.e., persistence) was not reduced tremendously by food restriction (Figure 4b), and these under-fed males apparently met more resistance from well-fed, sexually mature females (Figure 5b), which they could not easily overcome. This latter outcome also seems more readily interpretable biologically than the decrease in shaking (S^*) predicted by the model (Figure 1d), which might conceivably result because copulation is delayed and older females are more eager and less resistant to copulate. In either case, the obtained outcome reflects the natural situation that mature females of this species ultimately mate every time they encounter a mate when they come to the dung to lay eggs and might therefore be taken as evidence that sexual conflict over copulation frequency has generally been resolved in favor of males (cf., Parker et al. 1993; Ding and Blanckenhorn 2002).

In setting up our experiment in the Introduction, we had conceived that larger females may be intrinsically more resistant and larger males intrinsically more persistent, thus affecting the baseline levels (Figure 1a,b), simply because they are stronger and/or more competitive. Contrary to this expectation, however, body size did not significantly influence the timing of mating, t^* , or the amount of shaking, S^* , in either study, a robust result. Large size relative to the mating partner also generally did not strongly affect mating outcome in a previous study of yellow dung flies (Ding and Blanckenhorn 2002). Body size may additionally affect the slope parameters r and p . For example, larger females or males might require

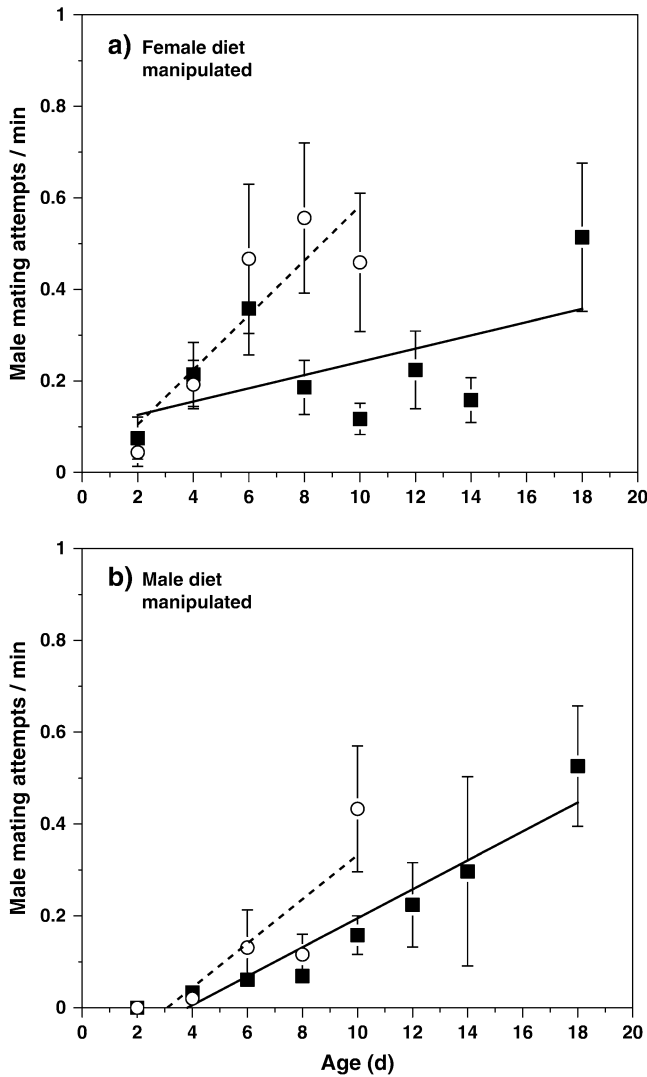


Figure 4
Male mating attempts per minute at high (open circles and dashed line) and low food (closed squares and solid line) when (a) female and (b) male diet was manipulated (Study 2). Linear fits are indicated.

more time to reach sexual maturity, predicting later copulation. However, Blanckenhorn and Henseler (2005) have shown that the effect of body size on physiological maturation is negligible. We conclude that yellow dung fly females and males show little systematic variation in resistance and persistence (respectively) relating to body size or at least seem to behaviorally compensate for their size in a way that eventually leads to the same outcome.

Nevertheless, we interpret the pattern of male mating attempts and female shaking found here as sexual conflict over when exactly a young female mates for the first time. In other words, female shaking can be interpreted as a signal to males that she is not yet receptive, to which males apparently respond at least initially (Figure 2). Once males have attempted mating, they may somehow be able to perceive if females are not yet mature, for example, by using contact pheromones as a possible cue. Our data show some evidence for this: whereas male persistence continuously increased with age in the male diet manipulation experiment (Figure 4b), in concert with their maturing sperm stores (Blanckenhorn and Henseler 2005), mating attempts in the female diet manipulation ex-

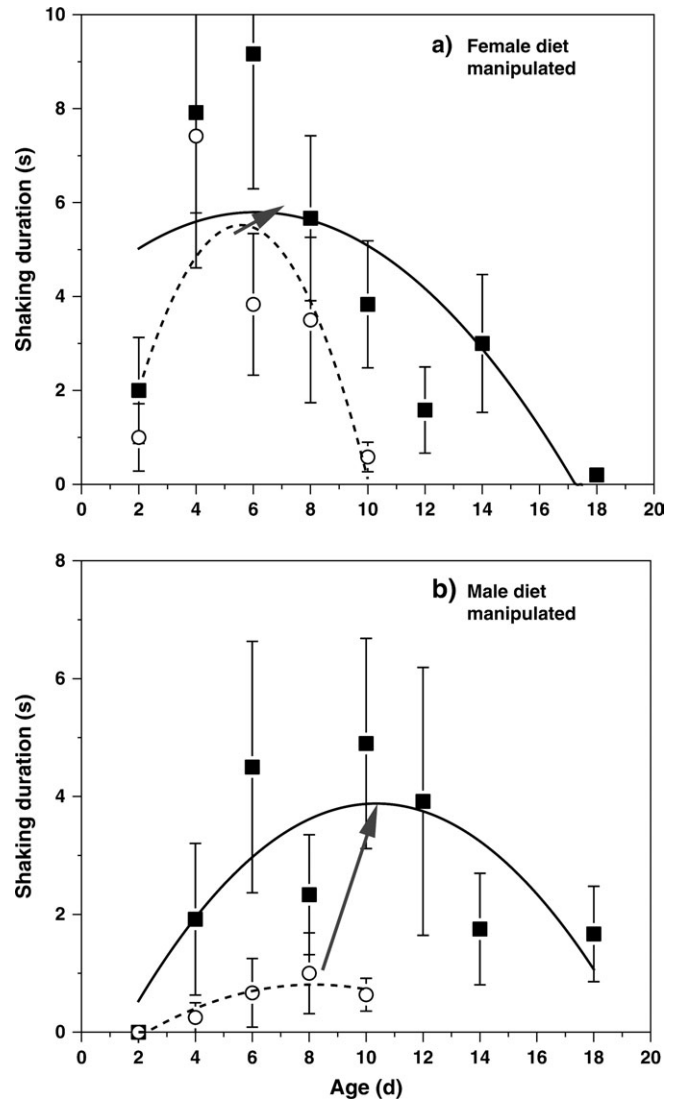


Figure 5
Cumulative female shaking duration at high (open circles and dashed line) and low food (closed squares and solid line) when (a) female and (b) male diet was manipulated (Study 2). Quadratic fits are indicated. Arrows show the shift in the maximum shaking, $S(t)$, with diet as predicted in Figure 1.

periment were quite frequent already when mature males were confronted with 4-day-old females but infrequent with the very youngest (2 days old) females (Figure 4a). The latter suggests that very young females are apparently unattractive for males even from a distance. However, males apparently cannot discriminate virgins from nonvirgins (Parker et al. 1993). In any case, if yellow dung flies indeed mate away from the cow pats (Parker et al. 1993), encounters of males with sexually immature females must occur commonly in nature.

Other than by some subtle means (Parker 1970c; Borgia 1981; Reuter et al. 1998), yellow dung fly females are believed to have only limited control over with whom they mate. In this species, mating is largely determined by the male, not least because males are larger (Ding and Blanckenhorn 2002), so they may be viewed as having “won” this sexual conflict, although there is growing consensus that it is generally difficult if not impossible to infer this from data for any single species (Crean and Gilburn 1998; Blanckenhorn et al. 2000; Chapman et al. 2003; Arnqvist and Rowe 2005). Nevertheless,

yellow dung fly females over evolutionary time have retained a shaking behavior common in many insect species to reject males (Thornhill and Alcock 1983; Rowe et al. 1994). This behavior has become subtle and may therefore be overlooked and has probably shifted function from an effective means of mate choice to a signal of unwillingness to the male (Ding and Blanckenhorn 2002). Even subtle shaking may be important in nature if females thereby can effectively escape unwanted male matings. Alternatively, or additionally, shaking may be an expression of female choice (Crean and Gilburn 1998; Blanckenhorn et al. 2000) or may relate to other yet unidentified mating cues (Eberhard 1996).

In summary, our simple model predicts when copulation should occur and how much female resistance (e.g., shaking) is expected as a function of age or time in various situations. In our simple version treated here, these predictions depend on 2 parameters in each sex affecting intrinsic (e.g., depending on body size or condition) or extrinsic (i.e., depending on the environment) female resistance and male persistence. Consequently, the qualitative predictions outlined in Table 1 can be tested via manipulations of the presumed underlying parameters (such as changes in food availability: Ortigosa and Rowe 2002). Given independent prior information on these parameters, even quantitative predictions can be generated. The model is applicable to multiple species and situations because sexual conflict is common in animals.

APPENDIX

We assume probabilistic exponential functions for female resistance, $R(t)$, and male persistence, $P(t)$, with Y -intercept parameters c_F and c_M ($c_F, c_M \geq 0$) and slope parameters r and p ($r, p > 0$). $R(t)$ starts at some baseline maximum and decreases monotonically toward a minimum (at infinity); analogously, $P(t)$ starts at some baseline minimum and increases monotonically toward a maximum (at infinity). Thus, the minimum and maximum (respectively) are never reached, so that a minimal level of resistance or persistence always remains (Figure 1):

$$R(t) = e^{-rt-c_F} \tag{1}$$

and

$$P(t) = 1 - e^{-pt-c_M}. \tag{2}$$

The baseline values are consequently $R(0) = e^{-c_F}$ and $P(0) = 1 - e^{-c_M}$, which are maximal and minimal (respectively) when c_F and c_M equal zero. When c_F and c_M exceed zero, this shifts the exponential functions toward the left (Figure 1), thus decreasing the initial baseline level of female resistance and increasing the initial baseline level of male persistence. $r, p > 1$ produce steeper and $r, p < 1$ shallower slopes (Figure 1). t denotes age or time and can be set independently for males and females. Thus, we assume that $R(t)$ and $P(t)$ start at some initial level, the 2 functions are monotonically decreasing and increasing with time (respectively), overlapping over the entire valid parameter space $t > 0$, and crossing at some point. The latter occurs whenever $R(0) > P(0)$, that is, when $e^{-c_F} + e^{-c_M} > 1$.

The probability of shaking as a function of time (age) resulting from a multiplicative interaction of female resistance and male persistence, $S(t)$, can now be obtained as the product of $R(t)$ and $P(t)$, yielding the function

$$S(t) = R(t) \cdot P(t) = e^{-t-r-c_F}(1 - e^{-pt-c_M}). \tag{3}$$

The time and duration of maximal shaking can then be determined by differentiating $S(t)$ with respect to t (using the

product rule), rearranging, and setting the result equal to zero:

$$\frac{dS}{dt} = S'(t) = e^{-t-r-c_F}((r+p)e^{-pt-c_M} - r) = 0. \tag{4}$$

This yields the time of maximal shaking (based only on the term within the parenthesis),

$$t^* = \frac{(\ln(\frac{p+r}{r}) - c_M)}{p}. \tag{5}$$

From this, the corresponding maximal shaking duration S^* can be calculated by substituting Equation 5 into Equation 3 and rearranging:

$$\begin{aligned} S^* = S(t^*) &= \left(e^{-\frac{r}{p}(\ln(\frac{p+r}{r}) - c_F) - c_M} \right) \left(\frac{p}{p+r} \right) \\ &= \left(\frac{p}{p+r} \right) e^{-c_F} e^{\frac{r}{p}c_M} \left(\frac{p+r}{r} \right)^{-\frac{r}{p}}. \end{aligned} \tag{6}$$

t^* is also the time at which this deterministic model predicts that copulation should occur. This is intuitive because at this point in time, the functions $R(t)$ and $P(t)$ cross (Figure 1) and male persistence becomes relatively stronger than female resistance. Note that qualitatively the same outcome would be predicted by an additive (as opposed to multiplicative) model, based on the same reasoning (cf., Figure 1). It is obvious that in a stochastic version of this model, the switch (from no copulation) to copulation at time t^* would be sigmoid instead of a step function, as would result from real data fraught with estimation error (see Figure 2).

From this formal treatment, we can derive the qualitative predictions with regard to changes in the intercept and slope parameters c_F, c_M, r , and p , which are summarized in Table 1; changes in the opposite direction are completely symmetrical. 1) When the initial female resistance decreases, that is, as c_F increases, shaking decreases, but the time of copulation and maximal shaking does not change (Figure 1a). The latter is an unintuitive result that derives from the formal exponential model because the time of maximal shaking t^* is independent of c_F (Equation 5). 2) When the baseline male persistence decreases, that is, as c_M decreases, shaking decreases but copulation occurs later (Figure 1b). 3) When female resistance declines more slowly with time, that is, as r decreases, shaking increases and copulation occurs later (Figure 1c). 4) Under most parameter values, when male persistence inclines more slowly with time, that is, as p decreases, shaking decreases and copulation occurs later (Figure 1d). However, under a small set of parameters (i.e., when $c_M > 0$), but which are not further formalized here, copulation can also occur sooner.

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