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Size-Dependent Mating Success at Various Nutritional States in the Yellow Dung Fly

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Abstract

Mating success not only depends on genetic quality, but also equally on environmental factors, most prominently food availability. We investigated the interactive effects of nutritional state and body size on mating success and copula duration in yellow dung fly males (Scathophaga stercoraria; Diptera: Scathophagidae) of three body size selection lines in the laboratory in both non-competitive (single) and competitive (group) situations. Adults require protein and lipids from prey to reproduce, as well as sugars as an energy source for reproductive activity. We expected mating success to decrease with time because of sperm depletion (sugar treatment) and/or energy shortage (water treatment) relative to the control, prey plus sugar treatment. Based on physiological scaling, we also expected large-line males to become depleted either sooner because of their higher energy and sperm demands, or later because of their more efficient energy use. Average mating success indeed declined over a period of 5–7 d (or 5–15 potential copulations per male), but equally for all food treatments and body size classes. Surprisingly, water-fed and small-line males had the highest mating probability in the non-competitive setting, while in the competitive setting large-line males had the highest success. Energy-depleted males showed apparent terminal investment. Small males acquired females more readily but eventually lost them to larger males in the competitive situation. As shown before, copula duration was inversely related to body size and increased with copulation number, independent of food treatment. We conclude that sugar or prey shortage has little effect on mating success in the short term, and does not differentially affect males of different sizes.

Introduction

In nature an individual’s mating success will depend on its genetic constitution (Andersson 1994) as well as on its nutritional or health state (Mangel & Clark 1988), and differentiating between these effects lies at the heart of the evolutionary study of sexual behavior (Bakker 1999). However, this is often not accounted for in laboratory studies, which typically feature rather affluent conditions, thus blurring this distinction and any interaction between genetic and environmental factors. For example, it is well known that good environments often mask expected life history trade-offs (Ojanen et al. 1979; van Noordwijk & de Jong 1986; Alatalo et al. 1990; Schluter et al. 1991; Rowe & Houle 1996). To get the full picture, reproductive success therefore needs to be investigated in several situations, particularly including restricted environments approximating natural conditions.
Body size is a prominent trait that generally positively affects reproductive success and behavior in many animals (Andersson 1994), to the extent that the disadvantages of large size often remain enigmatic (Blanckenhorn 2000, 2005). In the physiological literature there are two contrasting hypotheses concerning the energetics of animals in relation to their body size. On the one hand, larger individuals have an absolutely higher energy demand for maintaining their body functions, which is expected to negatively affect an individual's fitness particularly in food-limited environments (Blanckenhorn et al. 1995; Wikelski et al. 1997; Donohue et al. 2002). On the other hand, large body size apparently confers more efficient energy use because mass-specific metabolic rate decreases (Kleiber's 1932 3/4-power law; Hemmingsen 1960; Heusner 1982; reviewed in Glazier 2005). The former hypothesis predicts faster, the latter relatively slower depletion of energy reserves in larger individuals. Moreover, larger individuals are expected to consume more food in order to meet their higher demands and consequently may have greater energy stores to begin with (Reim et al. 2006; Blanckenhorn et al. 2007). Depending on the availability of nutrients in the environment, these basic physiological effects should ultimately also affect an individual's success in acquiring mates.

The yellow dung fly Scathophaga stercoraria (Diptera: Scathophagidae; sometimes Scatophaga) is a classic species for studies of sexual selection (Parker 1978; Simmons 2001). Larger body size predicts higher male mating success in the field and in the laboratory, which is largely mediated by male–male competition, including mate take-overs by fights (Borgia 1980; Sigurjónsdóttir & Snorrason 1995; Otronen 1995; Jann et al. 2000; Simmons 2001; Blanckenhorn et al. 2003a,b). Larger body size is also associated with shorter copula duration in this species, because larger males supposedly transfer more sperm per unit time and have higher chances of obtaining further mates (Parker 1970; Ward & Simmons 1991; Parker 1992; Parker & Simmons 1994, 2000; Hellriegel & Ward 1998; summarized in Simmons 2001). Yellow dung fly larvae are coprophagous, feeding on and developing in dung of large mammals (primarily cows), while adults are sit-and-wait predators of small insects that also imbibe nectar and fresh dung (Hammer 1941). Adults are nutritionally anautogenous, repeatedly requiring protein and lipids from fresh prey to produce eggs and sperm (Foster 1967), although they can subsist for long time on water and sugar or nectar (their energy source) without reproducing (W. U. Blanckenhorn, pers. obs.). Ward & Simmons (1991) showed that S. stercoraria males that had access to prey had full testes, allowing them to copulate with at least five females. Larger males apparently require more prey (Blanckenhorn & Viele 1999). Otronen (1995) and Blanckenhorn et al. (2003a) found that in the field male mating success is positively associated with energy reserves. However, beyond these broad generalizations the mechanisms underlying the relationship between nutrition and mating success has not been studied in detail in yellow dung flies.

In this laboratory study we investigated the effect of nutrition on mating success in yellow dung fly males. We worked with yellow dung flies artificially selected for large and small body size in the laboratory for 18 generations (plus unselected control lines based on random mating; see Teuschl et al. 2007), which here primarily served to augment the available body size range. After having been initially fed upon emergence with all necessary nutrients (water, sugar, Drosophila prey) so that they could become sexually mature, males of all three size classes were randomly assigned to one of three food treatments: (1) water only, (2) sugar and water, and (3) Drosophila prey, sugar and water ad libitum (control). The males had continuous access to these nutrients over the entire duration of the experiment. Relative to the continuously well-fed controls, we expected the water and the sugar treatment to eventually reduce copulation success because of energy plus sperm and sperm depletion, respectively (Foster 1967; Ward & Simmons 1991; Blanckenhorn & Henseler 2005). We further expected this depletion effect to interact with body size, depending on which of two contrasting physiological hypotheses above exerts the dominant net effect. In particular, based on results obtained for teneral (i.e. freshly emerged) adults by Reim et al. (2006) and Blanckenhorn et al. (2007), large-sized males were expected to survive and be reproductively active for longer because they have more energy reserves and use these reserves more efficiently, thus supporting the relative efficiency hypothesis. We tested individuals singly, thereby assessing mere physiological effects on an individual's capacity to mate, as well as in a more natural competitive group situation, additionally allowing energy being spent in male–male behavioral interactions (cf. Blanckenhorn et al. 2003b).

**Methods**

All yellow dung flies used for this experiment stemmed from our small, control or large laboratory
selection lines (Teuschl et al. 2007). There were originally two replicates per line, which were crossed two generations before our experiments to offset any possible inbreeding effects. To generate the test flies, females of all three lines (small, control, large) were allowed to mate within the line and lay eggs. Full clutches were transferred into containers with unlimited dung (>2 g per larva) using standard rearing methods (Amano 1983) and held at 20°C. Upon emergence, all adult flies were kept singly in 100 ml glass bottles with moistened cotton, sugar and *Drosophila melanogaster* prey *ad libitum*. Females reach sexual reproduction after about 10 d and continued to be fed *ad libitum*. Males were fed for 5–7 d, at which time they are sexually mature and should have produced sperm (Blanckenhorn & Henseler 2005); thereafter they were randomly allocated to one of the three food treatments specified above. Our copulation tests described below started on day 3 after start of the food treatment (acclimation period).

For the non-competitive experiment using single flies, we transferred males of a given size class and food treatment into a new bottle containing a smear of dung and added a randomly picked, well-nourished, fertile female. We simply scored whether copulation occurred within the next 30 min, indicating willingness and ability of a male to reproduce, and if so noted copulation duration. After 30 min or when copulation had ended, both individuals were transferred back to their holding bottle. Each male was so tested on seven consecutive days. Up to 10 individual males could be tested in parallel random blocks. In total, there were 18 replicates per treatment combination.

For the competitive experiment, we assembled groups of nine males, one per size class and food treatment combination, into well-aerated transparent plastic boxes approximately 15 × 15 × 25 cm in size. The boxes had a screen door on one side and contained a small dung pat, approx. 12 cm in diameter, on a large piece of filter paper. For individual identification, all males were marked with numbered bee tags on their thorax. After the males had settled, we always added nine (unmarked) females within a short time interval of several minutes, at which time the observation period began. This 1:1 sex ratio in principle allowed each male to obtain a female, guaranteeing a high probability of copulating and hence sperm use for most males (cf. Blanckenhorn et al. 2003b). However, in practice several females were not mated, e.g. because they were hiding, thus guaranteeing some degree of competition; i.e. effectively the operational sex ratio was somewhat male-biased, as under natural conditions. After approx. 10–15 min of subsequent pairing activity, we simply scored which male had obtained a female (start value). After a further 30–45 min, when some reshuffling or take-overs might have occurred and some further males could have obtained a female, we scored again (end value). After a total trial period of approx. 1 h we removed all females. We did not score copula duration, which typically lasts 30–40 min (see Results). The same male group remained together (without food) and was tested three times on any given day, with 1–2 h between trials. The groups thus received three sets of nine females, so the maximal number of copulations per male was three per day. At the end of the day, the males were transferred back into their individual holding (and feeding) bottles. Up to three groups could be observed in parallel. Similar to the non-competitive experiment above, the same groups were tested on five consecutive days. In total, 10 groups (replicates) were so tested in randomized blocks over a period of 3 wk, so a given male could have copulated maximally 15 times.

For both experiments, the length of the right hind tibia of each male was measured at the end using a binocular microscope. As our focus was on male behavior, females could be reused in several trials with other males in a random fashion. Males that died over the course of the experiment were simply excluded from the final analysis in the non-competitive (single) experiment; if this happened in the competitive (group) experiment, the number of females added was reduced and mean mating success statistically corrected accordingly.

**Results**

As expected, based on our successful artificial selection (Teuschl et al. 2007), males of the three selection lines differed in body size (two-way ANOVA of hind tibia length with selection line and food treatment as fixed factors: $F_{2,150} = 66.19, p < 0.001$), but there were no size differences among the three food treatments, nor an interaction ($p > 0.2$; Fig. 1a).

**Non-Competitive Experiment**

The probability of males mating was analyzed using logistic regression for binary response variables with individual identity as a random effect, which is equivalent to a repeated-measures generalized linear model (GLM; cf. Competitive Experiment below)
with selection line and food treatment as fixed factors and test day as the repeated measure. Mating probability expectedly decreased with day ($\chi^2 = 11.83$, $p = 0.001$) as males depleted their energy and/or sperm reserves, but this occurred equally for all size classes (day-by-line interaction: $\chi^2 = 2.24$, $p = 0.327$) and all food treatments (day-by-food interaction: $\chi^2 = 1.57$, $p = 0.457$; day-by-line-by-food interaction: $\chi^2 = 9.15$, $p = 0.067$; Fig. 2). Surprisingly, water-fed males showed the highest overall mating probability [82.4 ± 3.29% (SE); prey-fed 73.2 ± 4.78%; sugar-fed 68.3 ± 3.86%; food effect: $\chi^2_{150} = 8.68$, $p = 0.013$; Fig. 2]. Equally surprisingly, small-line males had the highest overall mating probability [82.9 ± 3.51% (SE); medium (control) 78.1 ± 4.02%; large 63.0 ± 4.23%; line effect: $\chi^2_{150} = 8.68$, $p = 0.040$; line by food interaction $p > 0.15$; Fig. 2].

Copula duration of a given male increased with day (or copulation number) from a mean of 36.57 ± 1.45 (SE) min (first day) to 43.24 ± 1.89 min (fourth day; repeated-measures GLM for the subset of 83 males that copulated four times: $F_{3,72} = 3.59$, $p = 0.018$; all interactions with line and food $p > 0.2$), a result previously reported by Ward & Simmons (1991). When analyzing an individual male’s mean copulation duration (over as many copulations as it had), copula duration was inversely proportional to body size line ($F_{2,147} = 3.42$, $p = 0.035$; food effect and interaction $p > 0.2$; Fig. 1b). When including hind tibia length as an additional covariate in the model, the
selection line effect disappeared, suggesting that the genetic effect of body size line is largely equivalent to the phenotypic effect of body size on copula duration typically obtained (e.g. Parker & Simmons 1994).

Competitive Experiment

We analyzed the number of mates obtained per day (between zero and three) using a doubly repeated-measures GLM with selection line and food treatment as fixed factors and test day and beginning vs. end value (see Methods) as repeated measures. Overall copulation success decreased with body size selection line [large 8.33 ± 0.53 (SE); medium (control) 6.57 ± 0.72; small 5.12 ± 0.35; F_{2,81} = 7.69, p = 0.001] but did not systematically vary among food treatments (F_{2,81} = 0.68, p = 0.503), nor was there an interaction (F_{4,81} = 1.72, p = 0.153). There was also no overall decrease of copulation success with day (F_{4,81} = 0.38, p = 0.897; Fig. 3). However, copulation success of water-fed males slightly declined while that of prey-fed males slightly increased with day (day-by-food interaction: F_{8,81} = 2.17, p = 0.030; Fig. 3). Furthermore, copulation success of large-line males remained high until day 5 while that of small-line males declined and only rebounded on day 5, with the control line being intermediate (day-by-line interaction: F_{8,81} = 2.27, p = 0.023; all other interactions p > 0.2; Fig. 3). Finally, systematic changes between the beginning and the end of a given group trial were apparent, equally for all food treatments: small males obtained copulations more quickly but were typically later partly supplanted by larger males (beginning/end-by-line interaction: F_{2,81} = 7.56, p = 0.001; all other interactions p > 0.2; Fig. 4).

Discussion

We found little evidence that mating success of yellow dung flies is affected by their nutritional state, at least in the short term. Average mating success did decrease over days in the non-competitive (single) experiment, so on average males must have suffered some energy and/or sperm depletion, but it did so equally in males held on water only, sugar, or sugar and prey (i.e. the well-fed control). In the competitive experiment mating success only decreased in the water-fed males, while it actually increased in the prey-fed males. We expected the control group to continuously perform well, as they continuously received sugar as energy supply as well as prey to replenish their sperm reserves, whereas the sugar-fed group should have become sperm-depleted, and the water-fed group should additionally have become energy-depleted. Sperm depletion is detectable after about five copulations (Ward & Simmons 1991), and in our experiments individual males had the opportunity to copulate up to seven times in the non-competitive setting or maximally 15 times in the competitive setting within 7 and 5 d, respectively. It is clear that water-fed males were energy-stressed, as many of them died soon after our experiment, and some even before, but this did not negatively affect their mating effort. To the
contrary, at least in the non-competitive situation water-fed males did better than those fed sugar and/or prey. This suggests some sort of terminal reproductive investment (Clutton-Brock 1984; Morrow et al. 2003): facing no possibility to find more food, males apparently invested all their remaining resources into mating to die soon thereafter.

In addition, body size did not clearly interact with nutrition in such a way as to indicate whether large or small individuals can last longer on their energy stored. Depending on whether large individuals have an absolute energy demand disadvantage or an efficiency advantage (in addition to their energy uptake and reserve advantage in absolute terms because of larger size: Blanckenhorn & Viele 1999; Reim et al. 2006), we expected mating success of large males to become depleted more quickly or more slowly with time, respectively (Kleiber 1932; Blanckenhorn et al. 1995; see Introduction). There was some indication in support of the latter, as at least in the competitive group situation performance of the large-line males was stable over time to only drop on the last day, whereas the performance of the small-line male dropped steadily to rebound only when the larger males weakened on day 5 (with control males being intermediate). However, this effect was not apparent in the non-competitive, single situation. It is possible but doubtful that these different size-dependent male characteristics or mating tactics are a property of our selection lines, perhaps due to a genetically correlated response, although this was not the central question here. More likely they are a general phenotypic effect that also occurs in field flies, as is suggested by the fact that the selection line effects disappeared when including hind tibia length as a covariate in our statistical model (see Results). Copula duration (only assessed in the non-competitive experiment) followed the plasticity usually seen in studies of yellow dung fly phenotypes: it increased with decreasing body size (Parker & Simmons 1994; Simmons 2001) and also increased with time (i.e. copulation number) in a given male (Ward & Simmons 1991), thus validating our results.

In summary, our study shows, somewhat surprisingly, that variation in nutrition or energy reserves does not strongly affect short-term male mating success in the yellow dung fly. Energy-stressed or sperm-depleted males of all sizes showed normal competitive mating behavior, at times approaching terminal investment. Of course, we did not measure fertilization success, which could be affected by low sperm numbers and perhaps even low energy reserves. Our results using reproductive individuals in a mating context therefore did not support the energy efficiency hypothesis, as was the case for starvation resistance of freshly emerged adults subsisting on their teneral energy reserves (Reim et al. 2006; Blanckenhorn et al. 2007). Perhaps the physiological advantages and disadvantages of large body size cancelled out in the situation tested here. We therefore conclude that size-dependent energetics play a minor role in determining the short-term reproductive success of yellow dung fly males.
Whether size-dependent advantages of large yellow dung flies perhaps become apparent under more realistic field conditions where individuals have higher energy demands because of longer traveling distances, and whether such effects generally occur in other animals or not (e.g. Moya-Laraño et al. 2007), remain to be shown.

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