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Alternative mating tactics in the yellow dung fly: resolving mechanisms of small-male advantage off pasture

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Recent work suggests that the yellow dung fly mating system may include alternative patroller–competitor mating tactics in which large males compete for gravid females on dung, whereas small, non-competitive males search for females at foraging sites. Small males obtain most matings off pasture, yet the behavioural mechanism(s) giving rise to this pattern are unknown. We investigated the male and female behaviours that determine mating success in this environment by conducting field mating experiments and found small males to benefit from several attributes specific to the off-pasture mating environment. First, small males from foraging sites exhibited higher mating propensity, indicating that large males away from dung may be depleted of energy and/or sperm. Second, small males were more discriminating, being significantly less likely to attempt with non-gravid females, which are absent on dung but common off pasture. Third, non-gravid females were generally more likely to actively struggle and reject mating attempts; however, such behaviours occurred disproportionately more often with large males. Female *Scathophaga stercoraria* thus appear to preferentially mate with small males when off pasture. These findings challenge assumptions about male–female interactions in systems with alternative mating tactics and reveal hidden processes that may influence selection patterns in the field.

1. Introduction

When relatively few (dominant) males in a population are able to monopolize mating opportunities with females, sexual selection is intense and unsuccessful (subordinate) males are predicted to adopt non-competitive, alternative mating tactics (AMTs) that bypass the traditional selection process [1–4]. For example, subordinate males may infiltrate harems aggressively defended by dominant males (sneak tactic) or intercept females attracted to dominant males or their resources (satellite tactic). Alternatively, subordinates may disperse from the traditional breeding ground where male–male competition is intense and instead seek mating opportunities elsewhere (patroller tactic). Whereas large body size or weaponry is often advantageous in competition, the smaller size of subordinates can actually increase their success in the alternative tactic by enabling greater mobility and lower detection by dominant males or females during mate capture attempts [5]. Although these subordinate males benefit by avoiding costs associated with resource defence and competition, they are thought to suffer greater costs as a result of decreased mating opportunities and increased levels of sperm competition [6,7]. These differences in perceived reproductive potential and sperm competition intensity between males of alternative ‘roles’ typically result in subordinate males allocating relatively more of their energy budget to some reproductive characteristics (e.g. testis size, sperm viability or ejaculate size) [8–10] while also being less discriminating than dominant males during mate selection [11].

With a focus on variation in intrinsic male reproductive investment and behaviour, an extensive body of theoretical and empirical investigations with

diverse taxa have revealed mechanisms underlying differential mating success of males adopting AMTs. Numerous studies have further quantified fitness consequences of AMTs, thus providing important insights into the evolutionary maintenance of phenotypic variation within males of the same species [2,3,12]. Considerably less attention has been given to the role of females in biasing the outcomes of male mating success, despite recognition that female preferences can have important implications in the evolution and maintenance of male AMTs [13–18]. In the traditional view of sexual selection, female choice and male–male competition are thought to reinforce one another, with females preferring competitively dominant males, thus forcing subordinate males to ‘steal’ fertilizations by circumventing female choice via the alternative tactic [19]. By favouring dominance, females are thought to receive direct benefits in the form of access to food, shelter, high-quality oviposition sites and/or protection from predators or harassment by other males, in addition to indirect benefits in the form of superior genes [20]. Although evidence of direct benefits provided by dominant males is common across taxa [19], support for indirect benefits remains scarce, and in some fish species non-territorial (subordinate) males are either of higher genetic quality or are thought to provide important genetic diversity to offspring [17,21]. A growing body of work also suggests that dominant males often inflict greater direct costs on females during courtship or mating [22–24], and when these costs to females outweigh benefits, conflict between intra- and intersexual selection can occur [25]. Moreover, female preferences may be context dependent if the cost–benefit relationship of resisting or mating with different male phenotypes changes with female energy reserves [26], reproductive state [27–29] or spatio-temporal environmental variation (e.g. female preferences change or are suppressed as male–male competition intensifies) [25,30,31]. Such a scenario may be expected to occur in patroller–competitor mating systems, in which distinct competitive and non-competitive (i.e. no direct agonistic encounters between males occur) mating sites exist simultaneously [32–34]. Female presence between these environments can depend on the state of ovarian development (i.e. oviposition sites contain only gravid females, whereas non-gravid females populate feeding sites), and thus receptivity between environments may differ as well [6]. Patroller–competitor mating systems therefore represent ideal systems to investigate female mating decisions and their influence on the mating success of males employing alternative tactics.

The yellow dung fly, *Scathophaga stercoraria*, is a model system of both pre- and postcopulatory sexual selection owing to the intense male–male competition and frequent female remating that characterizes its mating system [35–38]. In brief, males gather on freshly deposited dung pats and compete for incoming gravid females, as dung is the exclusive oviposition and larval substrate for *S. stercoraria* [39]. The operational sex ratio is heavily male-biased and large males have numerous competitive and reproductive advantages that have probably generated the observed male-biased sexual size dimorphism [38,40]. Females arriving at dung pats are either captured by ‘floater’ males in the grass surrounding the dung or by males upon landing on its surface [41–43]. Males provide no courtship and females are thought to have little or no control in determining mates, although Borgia [44] suggests that females sometimes preferentially orient towards larger males when approaching the pat. By pairing with the largest males, Borgia [44] found that females were

better protected from costly takeover attempts of rival males which frequently occur throughout copula and oviposition [39,42,45]. Once oviposition is complete, females leave dung to feed and produce their next egg clutch.

Although copulations away from dung were initially thought to be rare and unimportant [37,46], recent work suggests they are frequent and that most females mate before ever arriving at dung [47]. Consistent with this finding, Pitnick *et al.* [45] discovered frequent copulations on composting apple fibre, or ‘pomace’, a feeding resource for livestock present on farms throughout the world [48–50] that doubles as a foraging substrate for many insects, including *S. stercoraria*. The socio-sexual environment of *S. stercoraria* on pomace differs from that on dung in several important respects: (i) pomace resource is much larger and less defensible; (ii) distribution of *S. stercoraria* is more dispersed (i.e. less densely populated); (iii) sex ratio is approximately equal; (iv) agonistic male–male interactions are absent; and (v) the vast majority of matings on pomace are obtained by a smaller size class of males typically not present on dung or in cattle pastures, despite males of all body sizes being abundant [45]. These findings indicate that the *S. stercoraria* mating system is characterized by alternative patroller–competitor mating tactics, although the underlying mechanism(s) giving rise to the small-male mating advantage at feeding sites are unknown. To explain this pattern, Pitnick *et al.* [45] proposed three non-mutually exclusive hypotheses. We have adapted and broadened these hypotheses into the following: (i) small males are specialized for the patroller tactic and more efficiently search for or capture females on pomace; (ii) females preferentially mate with small males (or are more resistant to mating with large males) in this non-competitive environment; and/or (iii) large males have lower mating propensity and/or are less persevering when females are resistant to mating. Reduced mating effort for large males is predicted on pomace owing to either the lower reproductive pay-off expected from extradung matings (females remate on dung, and the last male to mate is thought to sire approx. 80% of offspring [36,46]) or depleted energy reserves from competing on dung.

Here, we test predictions of these alternative hypotheses for the mating advantage of smaller *S. stercoraria* males in the pomace foraging environment. Mating trials were conducted in the field on dung and pomace, using discrete large- and small-male size classes in a fully factorial design. We quantified male mating attempts, female resistance behaviours and the outcome of male–female interactions, in addition to measuring morphological and physiological traits associated with the observed behaviours and mating outcomes.

2. Material and methods

(a) Mating trials and data collection

All experimental trials were conducted at Toad Hollow Farms, Nedrow, NY, USA from October to November of 2011 and 2012, which is approximately the duration of the autumn reproductive season in our population. Experimental adult *S. stercoraria* of both sexes were collected from composting apple pomace each day immediately before mating trials began and were stored in plastic vials until their use, thus ensuring that body sizes and physiological states of all individuals reflected natural conditions of the foraging site environment. Male *S. stercoraria* were separated into small and large groups by eye based on experience (but later measured; see

Table 1. Percentages of outcomes for all binary response variables are shown for female egg development and mating resource at each level of male size. (Total sample size for each category combination is shown in parentheses. Values for struggles and displacements (i.e. proportion of struggles resulting in displacement) include data from only 2012.)

	male size	non-gravid	gravid	dung	pomace
attempt	small	61 (128)	81 (31)	64 (56)	65 (103)
	large	45 (140)	53 (30)	42 (57)	49 (113)
successful attempts	small	73 (78)	92 (24)	83 (35)	75 (67)
	large	31 (59)	80 (15)	50 (22)	37 (52)
captures	small	85 (78)	92 (24)	91 (35)	84 (67)
	large	58 (59)	87 (15)	73 (22)	60 (52)
struggles	small	54 (35)	22 (18)	43 (30)	43 (23)
	large	76 (21)	67 (6)	73 (15)	75 (12)
displacements	small	22 (19)	0 (4)	23 (13)	10 (10)
	large	50 (16)	0 (4)	45 (11)	33 (9)

below) and intermediate males were discarded. The small-male class was meant to encompass those males that avoid dung and instead primarily adopt the patroller tactic, whereas large males reflected body sizes of that typically observed on dung [45].

Each trial entailed observation of a focal male released into a clear Plexiglas chamber with a single female. Chambers measured 19.1 L × 18.4 W × 14.0 H cm, thus allowing room for individuals to behave and move about (e.g. fly) in a largely unrestricted manner. The bottom of each arena was either packed approximately 2.5 cm deep with apple pomace or else contained an 'experimental' dung pat. These pats were formed from dung freshly collected (and noted to have been occupied by numerous *S. stercoraria*) in an adjacent pasture, and then formed into a pat on a circular plastic lid (14.6 cm diameter). Trials on pomace were conducted in 2011 ($n = 141$) and 2012 ($n = 88$) and were conducted with the chambers sitting immediately adjacent to the pomace pile. Trials on dung ($n = 116$) were conducted in 2012; in this case, chambers were placed approximately 25 m from the pomace pile.

For each trial, one haphazardly chosen female was aspirated into the chamber and left to acclimate for 5 min. Either a small or large male (alternating every trial) was then aspirated into the chamber and continuously observed for up to 30 min. In 2011, we then recorded whether a mating attempt was made, the latency to attempt and its outcome, which could fall into one of four categories: (i) 'female decamp'—male mating attempt was unsuccessful owing to the female flying away immediately upon contact (but prior to genital contact); (ii) 'male miss'—attempt was made but no contact occurred between the male and female, despite an absence of female avoidance behaviour; (iii) 'displacement'—female escapes male grasp via shaking, kicking, wing beating and/or rolling; or (iv) 'successful copulation'. If resistance occurred upon contact, we observed the pair until the struggle ended before classifying the attempt as a successful copulation or displacement. Trials were ended after outcomes were recorded. In 2012, the same design was used with two changes: (i) the duration of female struggles occurring immediately following capture was recorded, and (ii) trials were not ended after a failed mating attempt. In the latter instance, attempt outcomes were recorded and trials were allowed to continue for their full duration (30 min), during which the same data were collected for subsequent attempts.

After trials were terminated, males and females were taken to the laboratory in individual plastic vials and frozen. We then measured the hind tibia length (HTL), a standard measurement of body size in *S. stercoraria* [51], of all individuals to the nearest 0.01 mm using the ocular reticule of a Leica MZ12 stereomicroscope at 32× magnification. Females were additionally dissected to score

status of ovarian development and were grouped into two categories: (i) non-gravid, which ranged from newly post-oviposition (or emerged) females to those with intermediate egg development; or (ii) gravid, represented by the presence of chorion and respiratory horns on the majority of the egg clutch.

Male size classes, as measured by HTL, were largely non-overlapping (small-male range: 2.28–2.97 mm, $n = 163$; large-male range: 2.91–3.97 mm, $n = 171$). Overlapping data points ($n = 5$) were removed, leaving a small-male range of 2.28–2.88 mm ($n = 159$, mean ± s.d. = 2.57 ± 0.152 mm) and large-male range of 3.06–3.97 mm ($n = 170$, 3.47 ± 0.203 mm). Additional data points not included in body size distribution were excluded upon finding that the small-male used in the trial was actually a female ($n = 2$), the focal male ($n = 3$) or female ($n = 2$) escaped after the trial or that an individual was heavily infected with an entomopathogenic parasitic fungus ($n = 4$).

(b) Data analysis

All statistical analyses were performed in JMP 10 (SAS Institute, Cary, NC, USA) or R v. 2.12.1 (R Core Team [52]). Multiple logistic regression analyses were used to test the effects of male size (small or large), ovarian development (non-gravid or gravid) and resource (pomace or dung) on all categorical response variables. Year (2011 or 2012) was also included as a covariate in models where applicable to test for between-year differences. Reduced models were obtained through backward, stepwise elimination by removing non-significant factors and interactions ($p > 0.1$). Quantitative response variables including attempt latency and struggle duration were analysed using standard least-squares regression and zero-inflated negative binomial regression, respectively. All logistic regressions were performed with only data from the first attempt of each male; later attempts were only used to obtain resistance durations when these data were not available for the first attempt ($n = 6$).

3. Results

(a) Factors influencing mating attempt likelihood

Analysis of male mating propensity, using multiple logistic regression, revealed that small males were significantly more likely to attempt copulations than were large males ($n = 329$, likelihood-ratio (L-R) $\chi^2 = 11.037$, $p = 0.001$; see the electronic supplementary material, table S1; table 1 and figure 1). The egg development status of the female further influenced the

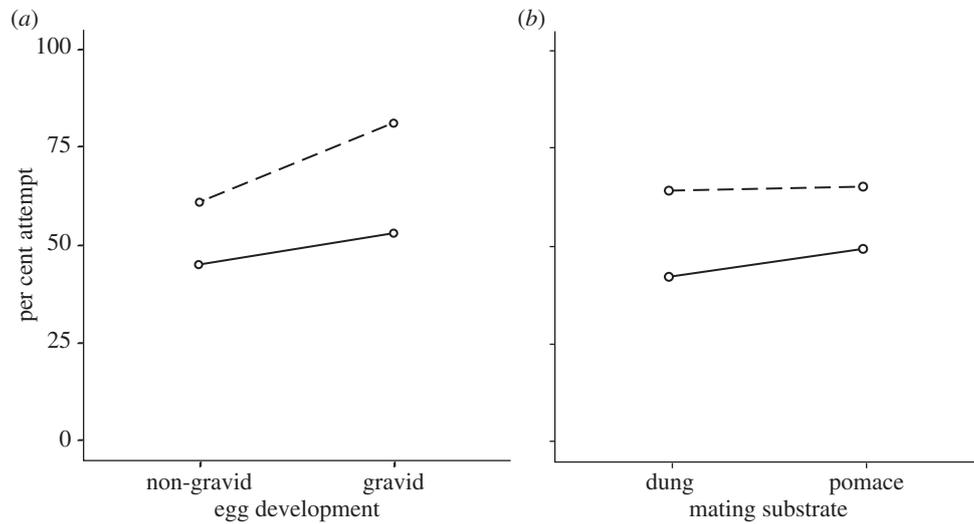


Figure 1. Interaction plots showing percentage of mating attempts by small (dashed line) and large (solid line) males with (a) non-gravid and gravid females and (b) on dung and pomace.

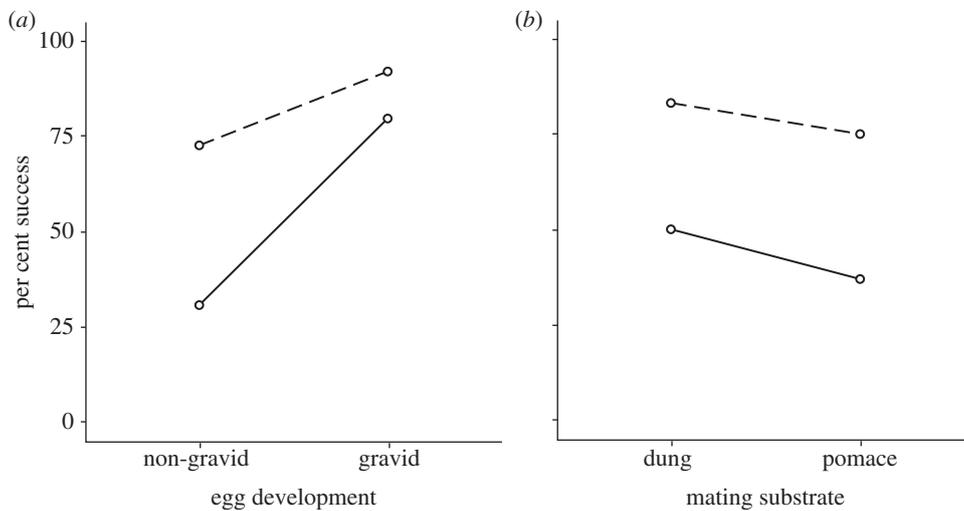


Figure 2. Interaction plots showing the percentage of mating attempts resulting in successful copulation by small (dashed line) and large (solid line) males with (a) non-gravid and gravid females and (b) on dung and pomace.

likelihood of male copulation attempt, with males attempting more often with gravid than with non-gravid females ($n = 329$, L-R $\chi^2 = 4.197$, $p = 0.041$; see the electronic supplementary material, table S1; table 1 and figure 1a). Although no significant interaction of male size class and egg development on attempt likelihood was present ($p > 0.1$), separate analyses focusing on male size classes independently revealed that the effect of egg development was significant within small males ($n = 159$, L-R $\chi^2 = 4.585$, $p = 0.032$; table 1 and figure 1a), but not for large males ($n = 170$, all effects $p > 0.1$; table 1 and figure 1a).

(b) Female resistance and mating outcomes

The most frequent outcome of mating attempts was copulation (60%, $n = 109$), whereas females successfully rejected 37% ($n = 67$) of male attempts (rejections: 61% decamp and 39% displacements). An additional 3% of trials ($n = 6$) were scored as 'male misses' with large males accounting for five out of the six. To broadly understand female influences on male mating success, we performed a multiple logistic regression with outcome of attempt (copulation or rejection—including both decamps and displacements) as a binary response variable

(male misses were excluded from this analysis). We found that small-male attempts were significantly more successful than large ones ($n = 176$, L-R $\chi^2 = 25.179$, $p < 0.001$; see the electronic supplementary material, table S1; table 1 and figure 2) and that the occurrence of rejections decreased significantly when the focal female was scored as having mature eggs ($n = 176$, L-R $\chi^2 = 14.681$, $p < 0.001$; see the electronic supplementary material, table S1; table 1 and figure 2a). There was also a trend for rejections to be more likely in 2011 than 2012, but this effect was not significant ($n = 176$, L-R $\chi^2 = 3.376$, $p = 0.066$; see the electronic supplementary material, table S1).

To better understand the greater success of small-male attempts in the context of our three hypotheses, we performed additional multiple logistic regression analyses using capture success (yes or no), likelihood of female resistance (yes or no) and male persistence (yes or no) as binary response variables. In each analysis, model effects were treated as described previously, although 'year' was not included as a covariate in the female resistance and male persistence analyses because relevant data were only recorded in 2012. The response variable for capture success included female decamps as 'no' and both copulation and displacement (in which males captured

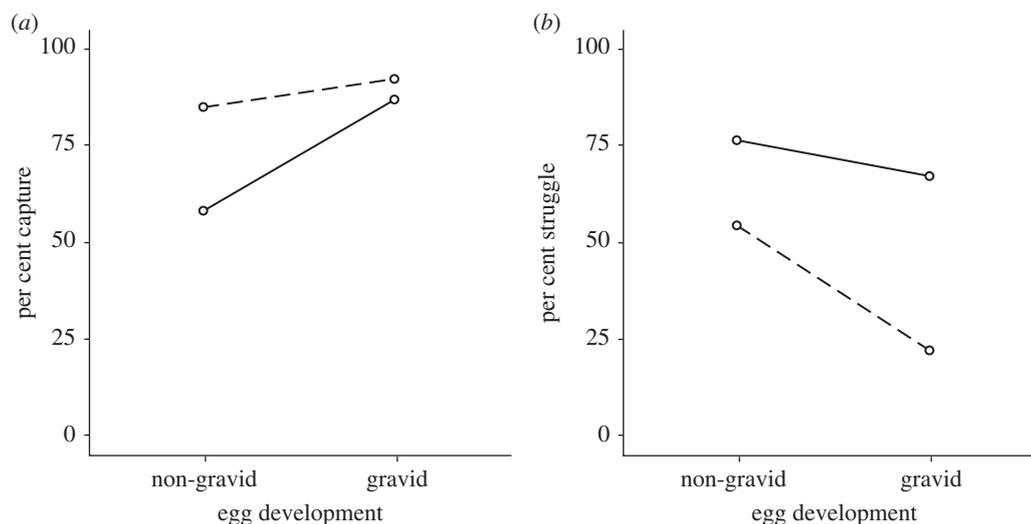


Figure 3. Interaction plots showing the effect of egg development on (a) capture success (i.e. percentage of attempts resulting in a successful capture) and (b) likelihood of female resistance (percentage of captures in which females struggled) by small (dashed line) and large (solid line) males.

females but were shaken off) were combined for the 'yes' category. Misses were again excluded to focus the analysis on failed captures driven by female avoidance behaviours. Small males had significantly higher capture success ($n = 176$, L-R $\chi^2 = 12.110$, $p = 0.001$; see the electronic supplementary material, table S1; table 1 and figure 3a), and non-gravid females, in general, were more likely to decamp than gravid females ($n = 176$, L-R $\chi^2 = 5.186$, $p = 0.023$; see the electronic supplementary material, table S1; table 1 and figure 3a). Next, the likelihood of female resistance was analysed using only trials in which males successfully captured females, thereby controlling for the possibility that decamps were influenced by male mobility rather than female choice. Both male size and female egg development significantly influenced the likelihood of a female struggle, with struggles disproportionately directed towards large males ($n = 80$, L-R $\chi^2 = 5.980$, $p = 0.015$; see the electronic supplementary material, table S1; table 1 and figure 3b) and performed by non-gravid females ($n = 80$, L-R $\chi^2 = 4.781$, $p = 0.029$; see the electronic supplementary material, table S1; table 1 and figure 3b). Finally, for our analysis of male persistence, which analysed the proportion of struggles resulting in displacement, no significant terms remained after backward, stepwise elimination; however, sample sizes were low and the absence of displacements by gravid females in 2012 (table 1) resulted in unstable parameter estimates. Separate χ^2 analyses using resource, male size and egg development as predictor variables were thus conducted, and similarly neither the effect of resource ($n = 43$, L-R $\chi^2 = 0.809$, $p = 0.369$; table 1) nor male size reached significance ($n = 43$, L-R $\chi^2 = 2.74$, $p = 0.098$; table 1). Struggles by non-gravid females were, however, more likely to result in displacement than those of gravid females ($n = 43$, L-R $\chi^2 = 5.91$, $p = 0.015$; table 1).

(c) Struggle duration and attempt latency

Our first analysis of a quantitative response variable focused on struggle duration. This dataset was restricted to successful copulation trials in which struggles either did not occur or failed because displacements limit the duration that females can struggle. Because zeros make up 54% of struggle durations ($n = 40$, range = 0–51 s), we checked the data for overdispersion by comparing their fit with a generalized linear model

(Poisson distribution and log link) to a zero-inflated negative binomial model. The zero-inflated model was a significantly better fit than the generalized linear model (Vuong's non-nested hypothesis test-statistic = 3.432, $p < 0.001$), and thus was used to account for overdispersion ($\theta = 1.791$). Egg development was found to be the only term with a significant effect on struggle duration; non-gravid females struggled longer than gravid females (estimate = -0.718 , s.e. = 0.347, $z = -2.070$, $p = 0.039$). Finally, standard least-squares regression was used to analyse latency to attempt matings and found small males to exhibit a significantly shorter latency than did large males (large: 13.11 ± 0.97 min, small: 9.65 ± 0.85 min, $F_{1,180} = 7.268$, $p = 0.008$).

4. Discussion

Descriptions of male–female interactions away from the primary breeding ground in patroller–competitor mating systems remain scarce for most species. Researchers have suggested that females might play a more important role in determining mating success in these non-competitive environments than at traditional breeding sites where female receptivity is high and preferences may be influenced or suppressed by male–male competition [6,53]. In yellow dung flies, the paucity of research focused on mating processes occurring off pasture in the foraging environment has long represented an important gap in our understanding of this model system [46]. Consistent with the field observations of Pitnick *et al.* [45], we found a small-male mating advantage for *S. stercoraria* collected from pomace and tested under non-competitive conditions, with 50% of small-male trials ending in a successful copulation, whereas large males were successful in approximately 18%. We show that the small-male advantage arises through a combination of male- and female-mediated behaviours that themselves are likely to be influenced by environmental or physiological conditions specific to the extra-dung environment. First, we found that small males collected from pomace have shorter latencies and are more likely to attempt copulations than large males collected from the same substrate, but surprisingly, neither latency nor attempt frequencies were affected by any proximate influence of the substrate the flies were tested on. These results indicate that

greater small-male mating propensity (subset of hypothesis iii) is perhaps the result of different energy demands and feeding opportunities associated with size-dependent AMTs, rather than calculations made by males regarding potential reproductive pay-offs of each mating location [46]. Large males presumably expend a great deal of energy competing on dung and are present on foraging resources only when energy reserves are low, whereas small (patroller) males have more consistent access to essential protein and sugars which are needed for sperm production and reproductive behaviours [54]. Depleted energy reserves have previously been shown to decrease male mating propensity, capture success and copulation duration in *S. stercoraria* [55,56], although in contrast to this study, Ward & Simmons [55] found a more pronounced decrease in mating propensity when energy depleted males were paired with females in the absence of dung. Alternatively, the attempt rate of large males in non-competitive environments may be inherently lower than that of small males, as suggested by Blanckenhorn *et al.* [57], who found lower mating success for larger males in the laboratory under non-competitive conditions, although it is unclear whether females influenced mating success in this experiment.

In this study, we found that females were frequently successful in resisting mating attempts (56 and 22% of large- and small-male attempts ended in rejection, respectively). Whereas the majority of female rejections took the form of decamps (61%), a substantive proportion of trials (39%) involved females physically displacing the males. Female resistance behaviours were often dramatic in form, including shaking, kicking, wing beating and/or rolling on the substrate. Nevertheless, resistant females were not always successful in dislodging the male and avoiding mating. The status of ovarian development was negatively related to the likelihood of resistance and rejection. Whereas rejections by gravid females were rare with both male size classes, this outcome occurred disproportionately more often in trials composed of non-gravid females and large males. These findings suggest that non-gravid females (which are most abundant on pomace; table 1) are playing an important role in generating the small-male mating bias observed by Pitnick *et al.* [45] at foraging sites. It should be noted, however, that our data do not permit fully disentangling the influence of active female preferences from differential capture success owing to a small-male mobility advantage or greater persistence by small males when faced with female resistance. Our design did, however, allow us to analyse the effect of male size on capture success, the proportion of captures in which resistance occurred and the likelihood that resistance by captured females successfully avoided copula (via displacement), and thus provided additional insight to the importance of each process. Our results are consistent with the hypothesis that small males predominantly adopt the patroller tactic and benefit from greater mobility in the capture of females (hypothesis i). Similar behavioural advantages of subordinate males occur in other species including *Onthophagus taurus* in which the greater manoeuvrability of small-horned males increased their ability to sneak copulations relative to large-horned males of similar body size [5]. The observation that five out of six male misses occurred in the large-male class could also be indicative of reduced aerial agility in competitor *S. stercoraria* males.

The hypothesis that females preferentially mate with small males (hypothesis ii) was also supported by a separate analysis

that controlled for the possibility of a small-male mobility advantage, by looking only at the likelihood of resistance for trials in which females were captured. Females were more likely to struggle when captured by large males, but displacement rates for males facing resistance did not significantly differ between size classes. To the extent that this outcome is indicative of male persistence, then the subset of hypothesis iii regarding size-dependent male perseverance is refuted. We thus interpret these findings as evidence of a female preference for small males. It should be noted, however, that our analysis of struggle likelihood excluded decamps, the most common form of female rejection, and thus, does not directly explain the full effect of differential rejection.

Female receptivity and resistance are commonly found to change with different stages of the reproductive cycle in clutch-laying species [58]. Previous work in *S. stercoraria* is consistent with this pattern, finding greater female resistance in non-gravid females, although reports of resistance have described the behaviour as gentle shaking or swaying and suggested that it probably does not function in mate choice [59,60]. In striking contrast to these earlier reports, we observed vigorous resistance, predominantly exhibited by non-gravid females, and provide evidence that such behaviour is non-randomly directed such that females discriminate against large males. The novelty of this finding may indicate important physiological differences between laboratory-reared and wild-caught populations or differences between isolated geographical populations, and these possibilities warrant additional research.

The adaptive significance of the observed female preference for small males is unclear. In the traditional view of AMTs, subordinate males are thought to be disfavoured in intersexual selection, and thus, by adopting an alternative tactic, bypass not only male–male competition but also female choice [19]. That is, subordinate males are typically perceived as ‘making the best of a bad situation’ (but see [61]). One possible explanation of our findings is that large-male copulations might inflict greater harm (direct costs) on females [62,63], creating a situation in which the cost of resistance is outweighed by the cost of mating with large but not small males. Evidence of increased harm imposed by dominant males is found in numerous taxa [22,24] and may be driving female preferences for subordinate males in other systems [23]. Alternatively, or additionally, female *S. stercoraria* might benefit from mating with small males by gaining genetic diversity or context-dependent survival advantages in offspring sired by genetically small males [64,65].

Our finding that gravid females were more likely to elicit mating attempts than non-gravid females may be attributable to males using female abdominal distention as a cue for attractiveness. Male choice based on indicators of female fecundity is common [66,67]. Within *S. stercoraria*, several lines of evidence suggest that male choice based on egg development or number occurs after contact [60,68] and, similar to our results, Blanckenhorn *et al.* [59] have shown that young females (with less developed eggs) were subjected to fewer mating attempts, indicating males assess ovarian status prior to contact. It is of consequence that this pattern of male mate choice in our study was primarily driven by small males, given the general assumption of AMT theory that patroller males have relatively few mating opportunities [46,55] and are simply making the best of a bad situation. In this view, small males should be expected to mate indiscriminately

when provided with an opportunity; however, if opportunities are abundant, as suggested by Pitnick *et al.* [45], males may increase reproductive gains per mating by choosing more gravid females. By doing so, male choice in this system may function as a sperm competition avoidance mechanism that minimizes female opportunities to remate prior to their return to dung.

Results of this study challenge assumptions about the roles of females and subordinate males in systems with AMTs. We stress the possibility that the discrete mating environments of patroller–competitor mating systems, differing both in competition intensity and the reproductive state of females, may frequently result in context-dependent behaviours (e.g. suppressed or plastic female preferences) that contribute to opposing patterns of selection. Theoretical work suggests that such variation in female preferences can have important implications for the maintenance of male AMTs [15,16,18] and this line of research is well suited to be addressed in *S. stercoraria*. However, understanding the impact that the patroller tactic has on the overall pattern and intensity of selection will first require understanding the frequency and reproductive pay-off of dung and off-pasture matings across

the spectrum of male sizes. Although these important components of fitness are currently unknown, we expect mating opportunities away from dung to be abundant and emphasize that future studies addressing these questions should be performed under natural conditions, given the important physiological differences of individuals that probably exist between environments. Work in other patroller–competitor systems should also be performed under conditions that reflect the natural environment and physiological states of individuals, as important insights into the mating system, otherwise cryptic, may be uncovered.

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References

1. Taborsky M, Oliveira RF, Brockmann HJ. 2008 The evolution of alternative reproductive tactics: concepts and questions. In *Alternative reproductive tactics: an integrative approach* (eds RF Oliveira, M Taborsky, HJ Brockmann), pp. 1–21. Cambridge, UK: Cambridge University Press.
2. Shuster SM, Wade MJ. 2003 *Mating systems and strategies*. Princeton, NJ: Princeton University Press.
3. Gross MR. 1996 Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol. Evol.* **11**, 92–98. (doi:10.1016/0169-5347(96)81050-0)
4. Wade MJ, Shuster SM. 2004 Sexual selection: harem size and the variance in male reproductive success. *Am. Nat.* **164**, E83–E89. (doi:10.1086/424531)
5. Moczek AP, Emlen DJ. 2000 Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: do alternative reproductive tactics favour alternative phenotypes? *Anim. Behav.* **59**, 459–466. (doi:10.1006/anbe.1999.1342)
6. Brockmann HJ. 2008 Alternative reproductive tactics in insects. In *Alternative reproductive tactics: an integrative approach* (eds RF Oliveira, M Taborsky, HJ Brockmann), pp. 177–223. Cambridge, UK: Cambridge University Press.
7. Parker GA. 1990 Sperm competition games: raffles and roles. *Proc. R. Soc. Lond. B* **242**, 120–126. (doi:10.1098/rspb.1990.0114)
8. Worthington AM, Gress BE, Neyer AA, Kelly CD. 2013 Do male crickets strategically adjust the number and viability of their sperm under sperm competition? *Anim. Behav.* **86**, 55–60. (doi:10.1016/j.anbehav.2013.04.010)
9. Kelly CD. 2008 Sperm investment in relation to weapon size in a male trimorphic insect? *Behav. Ecol.* **19**, 1018–1024. (doi:10.1093/beheco/arm058)
10. Simmons LW, Tomkins JL, Hunt J. 1999 Sperm competition games played by dimorphic male beetles. *Proc. R. Soc. Lond. B* **266**, 145–150. (doi:10.1098/rspb.1999.0614)
11. Amundsen T, Forsgren E. 2003 Male preference for colourful females affected by male size in a marine fish. *Behav. Ecol. Sociobiol.* **54**, 55–64. (doi:10.1007/s00265-003-0593-4)
12. Oliveira RF, Taborsky M, Brockmann HJ. 2008 *Alternative reproductive tactics: an integrative approach*. Cambridge, UK: Cambridge University Press.
13. Hugie DM, Lank DB. 1997 The resident's dilemma: a female choice model for the evolution of alternative mating strategies in lekking male ruffs (*Philomachus pugnax*). *Behav. Ecol.* **8**, 218–225. (doi:10.1093/beheco/8.2.218)
14. Luttbegg B. 2004 Female mate assessment and choice behavior affect the frequency of alternative male mating tactics. *Behav. Ecol.* **15**, 239–247. (doi:10.1093/beheco/arh002)
15. Alonzo SH, Warner RR. 2000 Female choice, conflict between the sexes and the evolution of male alternative reproductive behaviours. *Evol. Ecol. Res.* **2**, 149–170.
16. Alonzo SH. 2008 Conflict between the sexes and alternative reproductive tactics within a sex. In *Alternative reproductive tactics: an integrative approach* (eds RF Oliveira, M Taborsky, HJ Brockmann), pp. 435–450. Cambridge, UK: Cambridge University Press.
17. Reichard M, Le Comber SC, Smith C. 2007 Sneaking from a female perspective. *Anim. Behav.* **74**, 679–688. (doi:10.1016/j.anbehav.2007.03.005)
18. Henson SA, Warner RR. 1997 Male and female alternative reproductive behaviors in fishes: a new approach using intersexual dynamics. *Annu. Rev. Ecol. Syst.* **28**, 571–592. (doi:10.1146/annurev.ecolsys.28.1.571)
19. Andersson MB. 1994 *Sexual selection*. Princeton, NJ: Princeton University Press.
20. Qvarnström A, Forsgren E. 1998 Should females prefer dominant males? *Trends Ecol. Evol.* **13**, 498–501. (doi:10.1016/S0169-5347(98)01513-4)
21. Garant D, Fontaine P-M, Good SP, Dodson JJ, Bernatchez L. 2002 The influence of male parental identity on growth and survival of offspring in Atlantic salmon (*Salmo salar*). *Evol. Ecol. Res.* **4**, 537–549.
22. Pitnick S, Garcia-Gonzalez F. 2002 Harm to females increases with male body size in *Drosophila melanogaster*. *Proc. R. Soc. Lond. B* **269**, 1821–1828. (doi:10.1098/rspb.2002.2090)
23. Ophir AG, Galef Jr BG. 2003 Female Japanese quail that 'eavesdrop' on fighting males prefer losers to winners. *Anim. Behav.* **66**, 399–407. (doi:10.1006/anbe.2003.2230)
24. Head ML, Hunt J, Jennions MD, Brooks R. 2005 The indirect benefits of mating with attractive males outweigh the direct costs. *PLoS Biol.* **3**, e33. (doi:10.1371/journal.pbio.0030033)
25. Wong BBM, Candolin U. 2005 How is female mate choice affected by male competition? *Biol. Rev.* **80**, 559–571. (doi:10.1017/S1464793105006809)
26. Luttbegg B, Towner MC, Wandesforde-Smith A, Mangel M, Foster SA. 2001 State-dependent mate-assessment and mate-selection behavior in female threespine sticklebacks (*Gasterosteus aculeatus*, Gasterosteiformes: Gasterosteidae). *Ethology* **107**, 545–558. (doi:10.1046/j.1439-0310.2001.00694.)
27. Penton-Voak IS, Perrett DI, Castles DL, Kobayashi T, Burt DM, Murray LK, Minamisawa R. 1999

- Menstrual cycle alters face preference. *Nature* **399**, 741–742. (doi:10.1038/21557)
28. Lynch KS, Stanelly Rand A, Ryan MJ, Wilczynski W. 2005 Plasticity in female mate choice associated with changing reproductive states. *Anim. Behav.* **69**, 689–699. (doi:10.1016/j.anbehav.2004.05.016)
29. Lea J, Halliday T, Dyson M. 2000 Reproductive stage and history affect the phonotactic preferences of female midwife toads, *Alytes muletensis*. *Anim. Behav.* **60**, 423–427. (doi:10.1006/anbe.2000.1482)
30. Jirotkul M. 1999 Operational sex ratio influences female preference and male–male competition in guppies. *Anim. Behav.* **58**, 287–294. (doi:10.1006/anbe.1999.1149)
31. Reichard M, Bryja J, Ondracková M, Dávidová M, Kaniewska P, Smith C. 2005 Sexual selection for male dominance reduces opportunities for female mate choice in the European bitterling (*Rhodeus sericeus*). *Mol. Ecol.* **14**, 1533–1542. (doi:10.1111/j.1365-294X.2005.02534.x)
32. Alcock J. 1994 Alternative mate-locating tactics in *Chlosyne californica* (Lepidoptera, Nymphalidae). *Ethology* **97**, 103–118. (doi:10.1111/j.1439-0310.1994.tb01033.x)
33. Alcock J. 1997 Competition from large males and the alternative mating tactics of small males of Dawson's burrowing bee (*Amegilla dawsoni*) (apidae, apinae, anthophorini). *J. Insect Behav.* **10**, 99–113. (doi:10.1007/BF02765477)
34. Alcock J, Houston TF. 1987 Resource defense and alternative mating tactics in the Banksia bee, *Hylaeus alcyoneus* (Erichson). *Ethology* **76**, 177–188. (doi:10.1111/j.1439-0310.1987.tb00683.x)
35. Parker GA. 1970 Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**, 525–567. (doi:10.1111/j.1469-185X.1970.tb01176.x)
36. Parker GA. 1970 Sperm competition and its evolutionary effect on copula duration in the fly *Scatophaga stercoraria*. *J. Insect Physiol.* **16**, 1301–1328. (doi:10.1016/0022-1910(70)90131-9)
37. Parker GA. 1970 The Reproductive behavior and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae). VII. The origin and evolution of the passive phase. *Evolution* **24**, 774–788. (doi:10.2307/2406557)
38. Jann P, Blanckenhorn WU, Ward PI. 2000 Temporal and microspatial variation in the intensities of natural and sexual selection in the yellow dung fly *Scathophaga stercoraria*. *J. Evol. Biol.* **13**, 927–938. (doi:10.1046/j.1420-9101.2000.00230.x)
39. Hammer O, Volsøe A. 1941 *Biological and ecological investigations on flies associated with pasturing cattle and their excrement*. Copenhagen, Denmark: Bianco Lunos Bogtrykkeri A/S.
40. Ding A, Blanckenhorn WU. 2002 The effect of sexual size dimorphism on mating behaviour in two dung flies with contrasting dimorphism. *Evol. Ecol. Res.* **4**, 259–273.
41. Borgia G. 1982 Experimental changes in resource structure and male density: size-related differences in mating success among male *Scatophaga stercoraria*. *Evolution* **36**, 307–315. (doi:10.2307/2408049)
42. Borgia G. 1980 Sexual competition in *Scatophaga stercoraria*: size- and density-related changes in male ability to capture females. *Behaviour* **75**, 185–206. (doi:10.2307/4534080)
43. Parker GA. 1970 The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae). II. The fertilization rate and the spatial and temporal relationships of each sex around the site of mating and oviposition. *J. Anim. Ecol.* **39**, 205–228. (doi:10.2307/2896)
44. Borgia G. 1981 Mate selection in the fly *Scatophaga stercoraria*: female choice in a male-controlled system. *Anim. Behav.* **29**, 71–80. (doi:10.1016/S0003-3472(81)80153-4)
45. Pitnick S, Henn KRH, Maheux SD, Higginson DM, Hurtado-Gonzales JL, Manier MK, Berben KS, Guptill C, Uy JAC. 2009 Size-dependent alternative male mating tactics in the yellow dung fly, *Scathophaga stercoraria*. *Proc. R. Soc. B* **276**, 3229–3237. (doi:10.1098/rspb.2009.0632)
46. Parker GA. 1992 Marginal value theorem with exploitation time costs: diet, sperm reserves, and optimal copula duration in dung flies. *Am. Nat.* **139**, 1237–1256. (doi:10.2307/2462339)
47. Parker GA, Simmons LW, Ward PI. 1993 Optimal copula duration in dungflies: effects of frequency dependence and female mating status. *Behav. Ecol. Sociobiol.* **32**, 157–166. (doi:10.1007/BF00173773)
48. Fu-you Y, Zhou-yue QI, Cai-feng LI, Xiang-dong Y. 2000 Analysis of nutrition compositions of dried apple pulp and evaluation of their potentiality for feed [J]. *J. Gans. Ag. Univ.* **3**, 018.
49. Joshi VK, Sandhu DK. 1996 Preparation and evaluation of an animal feed byproduct produced by solid-state fermentation of apple pomace. *Bioresour. Technol.* **56**, 251–255. (doi:10.1016/0960-8524(96)00040-5)
50. Villas-Bôas S, Esposito E, de Mendonça M. 2003 Bioconversion of apple pomace into a nutritionally enriched substrate by *Candida utilis* and *Pleurotus ostreatus*. *World J. Microbiol. Biotechnol.* **19**, 461–467. (doi:10.1023/A:1025105506004)
51. Simmons LW, Ward PI. 1991 The heritability of sexually dimorphic traits in the yellow dung fly *Scathophaga stercoraria* (L.). *J. Evol. Biol.* **4**, 593–601. (doi:10.1046/j.1420-9101.1991.4040593.x)
52. R Development Core Team. 2011 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
53. Fincke OM. 1997 Conflict resolution in the Odonata: implications for understanding female mating patterns and female choice. *Biol. J. Linn. Soc.* **60**, 201–220. (doi:10.1111/j.1095-8312.1997.tb01492.x)
54. Foster W. 1967 Hormone-mediated nutritional control of sexual behavior in male dung flies. *Science* **158**, 1596–1597. (doi:10.1126/science.158.3808.1596)
55. Ward PI, Simmons LW. 1991 Copula duration and testes size in the yellow dung fly, *Scathophaga stercoraria* (L.): the effects of diet, body size, and mating history. *Behav. Ecol. Sociobiol.* **29**, 77–85. (doi:10.2307/4600588)
56. Otronen M. 1995 Energy reserves and mating success in males of the yellow dung fly, *Scathophaga stercoraria*. *Funct. Ecol.* **9**, 683–688. (doi:10.2307/2390161)
57. Blanckenhorn WU, Birrer M, Meier CM, Reim C, Teuschl Y, Weibel D. 2008 Size-dependent mating success at various nutritional states in the yellow dung fly. *Ethology* **114**, 752–759. (doi:10.1111/j.1439-0310.2008.01521.x)
58. Ringo J. 1996 Sexual receptivity in Insects. *Annu. Rev. Entomol.* **41**, 473–494. (doi:10.1146/annurev.en.41.010196.002353)
59. Blanckenhorn WU, Arthur BI, Meile P, Ward PI. 2007 Sexual conflict over copula timing: a mathematical model and a test in the yellow dung fly. *Behav. Ecol.* **18**, 958–966. (doi:10.1093/beheco/arm067)
60. Parker GA. 1970 The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae). V. The female's behaviour at the oviposition site. *Behaviour* **37**, 140–168. (doi:10.1163/156853970X00277)
61. Shuster SM, Wade MJ. 1991 Equal mating success among male reproductive strategies in a marine isopod. *Nature* **350**, 608–610. (doi:10.1038/350608a)
62. Johnstone RA, Keller L. 2000 How males can gain by harming their mate: sexual conflict seminal toxins and the cost of mating. *Am. Nat.* **156**, 368–377. (doi:10.1086/303392)
63. Arnqvist G, Rowe L. 2005 *Sexual conflict*. Princeton, NJ: Princeton University Press.
64. Reim C, Teuschl Y, Blanckenhorn WU. 2006 Size-dependent effects of larval and adult food availability on reproductive energy allocation in the yellow dung fly. *Funct. Ecol.* **20**, 1012–1021. (doi:10.1111/j.1365-2435.2006.01173.x)
65. Teuschl Y, Reim C, Blanckenhorn WU. 2007 Correlated responses to artificial body size selection in growth, development, phenotypic plasticity and juvenile viability in yellow dung flies. *J. Evol. Biol.* **20**, 87–103. (doi:10.1111/j.1420-9101.2006.01225.x)
66. Edward DA, Chapman T. 2011 The evolution and significance of male mate choice. *Trends Ecol. Evol.* **26**, 647–654. (doi:10.1016/j.tree.2011.07.012)
67. Bonduriansky R. 2001 The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol. Rev.* **76**, 305–339. (doi:10.1017/S1464793101005693)
68. Parker GA, Simmons LW, Stockley P, McChristie DM, Charnov EL. 1999 Optimal copula duration in yellow dung flies: effects of female size and egg content. *Anim. Behav.* **57**, 795–805. (doi:10.1006/anbe.1998.1034)