**Size-dependent ejaculation strategies and reproductive success in the yellow dung
fly, *Scathophaga stercoraria***

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1 Theory predicts that sperm competition will favor the production of larger ejaculates. However,
2 because the benefits of greater reproductive investment are balanced by the costs of
3 spermatogenesis, expenditure should depend on male physiology, mating rate and the
4 relationship between additional investment and fertilization gains. In the yellow dung fly
5 *Scathophaga stercoraria*, males adopt size-dependent alternative mating tactics that are
6 associated with discrete ecological resources (foraging and oviposition substrates), though males
7 switch between these environments throughout their lives. By copulating on foraging substrate
8 (fruit or flowers), males can bypass intense mate competition that occurs at oviposition sites
9 (cow dung), but as a consequence, must occupy a disfavored mating role (i.e., face a greater risk
10 that their ejaculate will be displaced from storage prior to fertilization). Small males often
11 specialize on foraging substrate, whereas large males only mate in this environment during
12 feeding bouts. Optimal ejaculate expenditure should therefore depend on male size and their
13 current mating role. By measuring copula duration (i.e., ejaculate expenditure) of natural matings
14 and assigning paternity to resulting offspring, we confirmed that copulations on dung sire
15 approximately three times as many offspring as those on foraging substrate. Further, large males
16 reduced copula duration on fruit, as predicted, since this strategy enables greater investment into
17 high pay-off matings on dung. Conversely, small males on dung copulated for shorter durations
18 than those found on foraging substrate, perhaps to minimize the risk of being displaced from
19 copula by a rival. These patterns of ejaculate expenditure translated into greater offspring
20 production for large males on dung and for small males on fruit. We discuss the possible
21 proximate factors driving these size- and context-dependent patterns of ejaculate allocation by
22 yellow dung fly males. Together, our findings shed light on the allocation strategies and
23 reproductive consequences of alternative mating tactics.

24 *Key words:* Alternative mating tactics, behavioral plasticity, male size, sexual selection, sperm
25 competition

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Post-copulatory sexual selection arises due to the co-occurrence of unrelated sperm within the female reproductive tract and is common in insects due to widespread polyandry and long-term sperm storage (Birkhead & Møller, 1993; Eberhard, 1996; Neubaum & Wolfner, 1999; Parker, 1970a; Simmons, 2001; Thornhill, 1983). Theory predicts that such selection should drive the evolution of larger ejaculates that more effectively dilute or displace sperm from previous matings (Parker, 1982, 1990a, 1993; Parker & Begon, 1993; Parker et al., 2010), and numerous comparative and experimental studies have supported this expectation across taxa (Gage, 1994; Harcourt et al., 1981; Hosken & Ward, 2001; Immler et al., 2011; Manier et al., 2010; Pitnick et al., 2001; Stockley et al., 1997). Increasing investment in spermatogenesis, however, comes with energetic costs that require males to extend foraging and development time (Ferkau & Fischer, 2006; Pitnick & Miller 2000; Pitnick et al., 1995), decrease mating rate (Dewsbury, 1982) and/or divert resources from other critical physiological processes (e.g., growth, immunity) to compensate (Parker & Pizzari, 2010; Scharf et al., 2013; Simmons, 2011). Selection should therefore favor life-history strategies that efficiently allocate resources and maximize lifetime reproductive success across naturally variable environmental conditions (e.g., risk of sperm competition, pathogen abundance, food availability).

Species in which males adopt status-dependent alternative mating tactics (AMTs) present a unique opportunity to study how different life-history demands shape resource allocation strategies. Dominant males generally compete with one another and defend females, which in turn favors the production of exaggerated weaponry or body size (Oliveira et al., 2008; Shuster & Wade, 2003). In contrast, subordinate males bypass pre-copulatory mate competition and, as a consequence, are forced into disfavored mating roles with reduced fertilization success per mating. For example, in sneak-guard mating systems, guard males only face sperm competition

when sneak males successfully infiltrate their territories, whereas sneak males encounter sperm competition from guards in essentially all matings. This tactic-specific variation in pre- and post-copulatory sexual selection intensity has driven divergent selection on male phenotype and resulted in male dimorphism (or trimorphism) in some species, where each phenotype is specifically associated with a mating tactic (e.g., small, weaponless males sneak and large males with weaponry guard; intermediate phenotypes are rare or absent) (e.g., Eberhard & Gutierrez, 1991; Kelly, 2005; Simmons et al., 2007b). Under these conditions, subordinate males generally divert resources from growth or the development of secondary sexual traits and instead disproportionately invest in testis size, which allows the production of larger, more competitive ejaculates that help compensate for their mating roles (Gage et al., 1995; Simmons & Emlen, 2006; Simmons et al., 1999b). This pattern of resource allocation is observed even when subordinate male mating rates are low (Neff et al., 2003). In contrast, little is known about resource allocation strategies in species where male behavioral tactics or the associated mating roles facultatively change throughout an individual's life (e.g., in response to male physiological condition or fluctuations in the local intensity of male-male competition), as is expected when male body size (or status) exhibits continuous variation (Taborsky, 1994, 1998; Waltz & Wolf, 1984). In this scenario, males may instead alter reproductive investment in a context-dependent manner based on their current mating role or physiological state (Bonduriansky, 2001; Lüpold, et al., 2011; Nicholls et al., 2001; Wedell & Cook, 1999; Wedell et al., 2002). However, allocation strategies and their reproductive consequences in such mating systems remain poorly understood.

Study system

In the yellow dung fly, *Scathophaga stercoraria*, males express size-dependent AMTs, as well as continuous variation in body size (i.e., status), and their mating roles are known to

91 change over time (Gress et al., 2014, 2016; Pitnick et al., 2009). For instance, “competitor”
92 males aggregate on fresh cow dung (i.e., the oviposition and larval substrate) where they engage
93 in intense competition to mate with females arriving to lay their clutch of eggs (Hammer, 1941;
94 Parker, 1970d). Larger body size better enables competitor males to defend their mates from
95 “takeovers” by rival males during protracted copulations (approximately 40 min) and the
96 subsequent oviposition phase (Borgia 1980, 1981; Ding & Blanckenhorn, 2002; Jann et al.,
97 2000; Otronen, 1995; Sigurjónsdóttir & Snorrason, 1995). Because of sperm displacement and
98 last-male sperm precedence, which have been well documented in *S. stercoraria*, successful
99 matings on dung result in high fertilization success since copula occurs just prior to oviposition
100 (Parker, 1970b; Simmons et al., 1996, 1999a). However, competitors also mate opportunistically
101 with non-gravid females between oviposition bouts at off-pasture feeding sites (e.g., flowers or
102 rotting fruit; Gress et al., 2014, 2016; Parker, 1970e, 1971; Pitnick et al., 2009). Using a marginal
103 value theorem approach, Parker (1992) found that competitors should reduce ejaculate
104 expenditure under these conditions to conserve resources for later matings on dung, where
105 fertilization success per sperm ejaculated is expected to be higher. Such a reduction in ejaculate
106 size (i.e., sperm number) can be achieved by simply shortening copula duration, since sperm
107 transfer rate is constant throughout mating in *S. stercoraria* (Parker et al., 1990).

108 In contrast, most small males avoid dung when male-male competition is intense. These
109 “patroller” males specialize in searching for females at off-pasture foraging sites where
110 competition is low or absent (Gress et al., 2014, 2016; Pitnick et al., 2009). Parker (1992)
111 predicted that patrollers would experience lower mating rates than competitor males based on the
112 observation that feeding resources (and foraging females) are more dispersed in their distribution
113 than dung pats. Based on this assumption, a second marginal value theorem model demonstrated

that patrollers should respond by increasing their ejaculate expenditure per mating opportunity (i.e., longer copula duration) because additional investment into the current female would result in greater fertilization gains than leaving to search for a new mate (Parker, 1992). However, for small males, specifically, this assumption is inconsistent with recent evidence showing that these males are largely excluded from mating on dung but frequently copulate at some feedings sites, in part because females are relatively more abundant and preferentially mate with small males in this environment (Gress et al., 2014; Pitnick et al., 2009). Moreover, other recently documented differences between mating environments may further influence size- or tactic-specific copula duration, including (1) the risk of being aggressively “taken over” by a rival during copula (Pitnick et al., 2009), (2) the status of female egg development, which influences both female attractiveness and resistance behavior (Gress et al., 2014) and (3) physiological constraints on ejaculate production (e.g., large competitor males may only visit foraging sites when depleted of ejaculate resources, as suggested in Gress et al., 2014).

In light of these discoveries, we here quantify the relationship between male size and ejaculate expenditure in both mating environments. Additionally, using previously developed microsatellite markers, we assigned paternity to offspring produced from these matings to assess the impact of male size and mating environment on reproductive pay-off.

METHODS

Size-dependent ejaculate expenditure and fertilization success associated with alternative mating roles

We collected male and female *S. stercoraria* of unknown age and mating history from off-pasture foraging sites located on the grounds of Toad Hollow Dairy Farm (Nedrow, NY, USA) throughout October and November 2013 and 2014. Foraging sites consisted of piles of apple pomace (approximately 2.5L x 1.5W x 1H m in size), which is the sugar-rich byproduct of cider production and is readily available on many farms in the region given its use as livestock feed (Gress et al., 2014, 2016; Pitnick et al., 2009). The location of this substrate on the farm was approximately 20 – 100 m from the nearest herd of cows, depending on which pasture the cattle were grazing. Effort was made to collect males from across the full range of body sizes in approximately equal proportions. Within 60 min from the time of collection, each male was randomly paired with a female in a clear plastic vial and placed on the pomace substrate where they were allowed to copulate to completion (until genital separation occurred). After copulations ended, pairs were separated to prevent additional mating and transported back to the laboratory where males were frozen and females were housed individually in a vial with *ad libitum* access to sugar water and *Drosophila* (Blanckenhorn et al., 2010). Once female abdomens appeared distended with mature eggs (usually 2-4 d after collection), they were transported back to Toad Hollow Farm and remated to a new, freshly collected male from dung. These copulations took place on fresh cow dung in the field, and experimental males were again specifically collected to represent the full range of body size variation present on this substrate. Immediately following their second experimental mating, females were transferred via aspirator into a plastic jar (40-dram tube, 49 x 85 mm; BioQuip Products, Rancho Dominguez, CA, USA) containing approximately 60 g of cow dung for oviposition, and all progeny were then reared to adulthood in the laboratory at 22°C (Blanckenhorn et al., 2010). All parental trios (consisting of a female and both of her experimental mates) and their offspring were frozen and stored at – 80°C

until paternity was assigned to offspring using four microsatellites (see *Paternity analyses*, below). This experiment, therefore, simulated the natural order of copulations and oviposition as they occur in the field, while also capturing natural variation in *S. stercoraria* condition, physiological state and behavioral tactics in each environment.

Copula duration was recorded for all matings on both substrates (pomace and dung) and used to assess ejaculate expenditure. Because sperm transfer rate (and therefore optimal copula duration) is known to vary with male size and temperature (i.e., larger body size and warmer temperatures result in faster sperm transfer and shorter copula duration; Simmons & Parker, 1992), we also recorded ambient temperature (to the nearest degree Celsius) in the field each day and later measured hind tibia length (HTL), which is a standard proxy of body size in *S. stercoraria* (e.g., Simmons & Ward, 1991), to the nearest 0.01 mm using a Leica MZ12 stereomicroscope at 32× magnification.

Paternity analyses

DNA was extracted from all experimental males ($N = 140$), and females ($N = 70$), as well as their offspring ($N = 12-16$ / parental trio) following the protocol of Demont et al. (2011). In brief, we cropped the head of each individual fly into one well of a 96-well plate that contained 100 μ l of 6% Chelex solution. Plates were then incubated at 58°C for 60 min, boiled at 100°C for 9 min, and cooled to 20°C. Plates were then refrigerated at 4°C for 24 hr and frozen for at least 24 hr at – 20°C. We then performed multiplex PCR to simultaneously amplify four microsatellite loci: SsCA3, SsCA16, SsCA24 and SsCA26 (Garner et al., 2000). Total PCR reaction volume was 10 μ l: 2 μ l of DNA/Chelex solution, 0.8 μ l of 10 μ M primer solution (containing forward and reverse primers for each of the microsatellite loci; all forward primers were directly labeled),

1.8 µl of Multiplex PCR 5x Master Mix (New England BioLabs), and 5.4 µl of molecular grade H₂O were added to each well of a new 96-well plate. PCR runs were performed using the protocol from Garner et al. (2000) with an annealing temperature of 60°C, and fragment analysis was conducted on the resulting product. Peaks for each individual were analyzed using GeneMarker Software (SoftGenetics®, State College, PA), and the genetic profiles of offspring were compared to those from its parental trio to identify alleles that would allow unambiguous discrimination of paternity or to rule out that either male was the sire. Since female mating history prior to the experiment was unknown, some low number of sperm from previous, non-experimental males may have remained in storage at the time of oviposition (Parker, 1970b).

Statistical analyses

We analyzed the effects of male HTL (mm), mating environment and their interaction on reproductive investment data using moderated multiple linear regression (SPSS v24, IBM, Armonk, NY). We repeated this analysis twice using two different response variables, and HTL was centered (mean = 0) in both models by subtracting mean HTL from each value. In the first analysis, we used raw copula duration as the response variable to assess time costs and allocation for small and large males in each environment. However, because sperm transfer rate increases with male body size, copula duration is not adequate for comparing total ejaculate expenditure or predicting paternity outcomes for males of different sizes – i.e., a one-unit change in copula duration for small males has less of an effect on the number of sperm ejaculated than a one-unit change for larger males. Therefore, we also estimated total sperm number per ejaculate using the known relationship between male HTL and sperm transfer rate (z/s): $z/s = 0.0018 * HTL^3 - 0.003$ (Parker & Simmons, 1991, 1994). Additionally, temperature was included in both models as a covariate to account for variation in copula duration and sperm number across days (Parker &

Simmons, 1991). Using sperm number as a dependent variable in this model, however, leads to the unusual scenario in which male HTL is both a predictor variable and part of the equation to estimate sperm number (dependent variable). For this reason, we present both models for comparison. Significant interactions were further probed using a “pick-a-point” approach at -1 SD and $+1$ SD of the mean (Aiken & West, 1991) using the macro MODPROBE (Hayes, 2001) to compare investment between environments for small and large males. Additionally, separate regression analyses of sperm number on male HTL were performed for pomace and dung matings to test for variation in ejaculate expenditure within each environment.

After identifying size-dependent patterns of ejaculate expenditure, we next analyzed whether these patterns influenced reproductive success by assessing the relationship between male HTL and paternity share using a generalized linear model with binomial distribution and logit link. Because males on dung are the last to mate before oviposition, their sperm should displace some proportion of those transferred during the previous mating on pomace and therefore result in greater fertilization success per sperm ejaculated. For this reason, we (1) performed separate regression analyses for each mating environment and (2) included sperm number of the last male to mate (dung male) as a covariate in the pomace model to account for its influence on pomace male fertilization success. When male HTL was significantly related to paternity outcomes, we then reran each model with focal male sperm number included as a covariate to determine whether these patterns were driven by size-dependent variation in ejaculate size or whether ejaculate “quality” differences or cryptic female processes may bias paternity in favor of small or large males (Snook, 2005). Finally, reproductive pay-offs of matings on dung and pomace were compared using a binomial generalized linear model. Two-tailed P values are reported throughout.

Ethical Note

This study complied with all USA laws regarding animal welfare, and no permits were necessary to conduct the experiment described above. All *S. stercoraria* used in this study were collected from the field using aspirators and gently transferred to vials. These flies were housed in the laboratory with *ad libitum* access to food and euthanized quickly by placing into a -20°C freezer.

RESULTS

We found highly significant interactions between male HTL and mating environment for both copula duration and sperm number (see Table 1, Fig. 1a & b for model comparisons). Copula duration decreased sharply with male HTL on pomace, but this pattern was less pronounced on dung (Table 1; Fig. 1a). Sperm number, which provides a standardized measure of ejaculate size, increased significantly with HTL for matings on dung but was unrelated to male size on pomace (linear regression, dung: $F_{2,62} = 21.4$, $R^2 = 0.389$, $P < 0.001$, pomace: $F_{2,62} = 1.331$, $R^2 = 0.01$, $P = 0.34$; Fig. 1b). Further analysis of these interactions demonstrates that small males (i.e., -1 SD from the mean) copulated for significantly longer durations on pomace than on dung (pick-a-point analysis: coefficient = -9.319 , $t = -2.021$, $P = 0.045$) and tended to transfer more sperm, though this effect was not significant (pick-a-point analysis: coefficient = -255.777 , $t = -1.55$, $P = 0.124$). Conversely, large males (i.e., $+1$ SD from the mean) reduced copula duration on pomace relative to dung (pick-a-point analysis: coefficient = 17.2 , $t = 3.96$, $P = 0.001$), which also resulted in significantly lower estimates of total sperm number per ejaculate (pick-a-point analysis: coefficient = 876.792 , $t = 5.64$, $P < 0.001$). As expected, based on patterns of sperm allocation, paternity share per mating increased significantly with male HTL on dung (Table 2,

model: *Dung* (a); Fig. 2a) but unexpectedly decreased with male HTL on pomace (Table 2, model: *Pomace* (a); Fig. 2b). The positive effect of male HTL on paternity share for matings on dung became non-significant after controlling for focal male sperm number (Table 2, model *Dung* (b)), whereas small males on pomace sired significantly more offspring than predicted based on their ejaculate expenditure (Table 2, model: *Pomace* (b)). On average, dung matings sired more than three times as many offspring than matings on pomace ($X \pm SD = \text{dung: } 0.71 \pm 0.192, \text{ pomace: } 0.23 \pm 0.177$; generalized linear model with binomial distribution and logit link: $z = -18.21, P < 0.001$).

DISCUSSION

Alternative male tactics in *S. stercoraria* are associated with different ecological resources. As expected, given that females always remate on dung prior to oviposition, we demonstrate that fertilization success also differs for copulations in each environment. On average, matings on pomace fertilized 23% of eggs in the subsequent clutch, whereas those on dung sired 71%. This disparity in per mating reproductive success between alternative mating environments may be even greater, as our experiment did not account for additional variation likely present in natural populations, including the occurrence of takeovers on dung or females mating with multiple males during a single inter-clutch feeding bout, both of which would further displace sperm transferred in extra-dung matings and reduce their average pay-off (Parker, 1970c, 1992). Nevertheless, this disparity in reproductive pay-off is predicted to drive differential ejaculate allocation strategies for males when moving from one substrate to the other. For large competitor males, who have relatively high mating success on dung, Parker (1992) concluded that copula

duration should be reduced when opportunistically encountering females while foraging, since their time and ejaculate resources would yield higher fertilization returns if invested on dung. Here, we confirmed this prediction in the field under natural conditions.

The proximate factor(s) responsible for the decreased expenditure on pomace remain(s) unclear. Ward & Simmons (1991) measured copula duration of laboratory-reared *S. stercoraria* males that (1) received varying levels of diet quality and (2) were either tested in the presence or absence of dung. This study found that the average length of copula *increased* in the absence of dung. However, this pattern did not result from ejaculate tailoring in response to the substrate but instead arose because poorly fed males (who copulated for shorter durations) were less likely to attempt copula when dung was absent. If competitor males only visit feeding sites when they are depleted of sperm or energetic reserves, as seems likely, then these physiological differences could explain the reduction in copula duration for large males on pomace. Consistent with this explanation, Gress et al. (2014) found that large males on pomace had significantly lower propensity to attempt copula than small males collected from the same substrate. Additionally, or alternatively, large competitor males could be tailoring their ejaculate size based on female abdominal distention (as in Parker et al., 1999), since *S. stercoraria* females on pomace generally contain immature ova and have flatter abdomens than those found on dung (Gress et al., 2014). Female fecundity and abdominal distention are widely used by males as indicators of female quality and influence male ejaculate expenditure across insect species (Bonduriansky, 2001).

Patterns of reproductive investment exhibited by small males were opposite of those observed in large males: small males found on pomace copulated longer than those found on dung. Since small males on dung are necessarily competitors, whereas those on pomace are likely patrollers (Gress et al., 2014, 2016), this pattern of differential investment most likely

indicates that optimal copula duration differs between alternative mating tactics. The direction of this change in small males, however, is not consistent with males tailoring their ejaculate size based on female abdominal distention since gravid females on dung should elicit greater expenditure (Bonduriansky, 2001, Parker et al., 1999). Similarly, because small-male mating success is low on dung (Borgia 1980; Borgia, 1981; Ding & Blanckenhorn, 2002; Jann et al., 2000; Otronen, 1995; Sigurjónsdóttir & Snorrason, 1995) but relatively high on pomace (Gress et al., 2014; Pitnick et al., 2009), trade-offs between mating rate and ejaculate size also fail to explain small-male ejaculation patterns. One explanation that warrants additional attention is that small males, who are most vulnerable to being taken over by a (larger) competitor male on dung, may reduce copula duration to minimize their risk of being displaced (Parker, 1970a; Pitnick et al., 2009). Numerous studies in other systems have previously demonstrated that the local competitive environment (e.g., presence of rivals) can cause males to alter their ejaculation strategy, including changes in the viability and number of sperm ejaculated in each mating (Candolin & Reynolds, 2002; Pound & Gage, 2004; Simmons et al., 2007a; but see Worthington et al., 2013). In the current study, we also demonstrated that, by decreasing copula duration on dung, small competitor males sired fewer offspring per mating than larger males on the same substrate. Nevertheless, this small-male strategy may be more advantageous than the alternative (i.e., prolonged copulation and increased risk of takeover) given the strong last-male sperm precedence pattern in this species (e.g., Parker, 1970c; current study).

Despite the reduction in small male copula duration on dung (relative to pomace), these males still copulated for longer durations than large males, a pattern previously reported numerous times in the laboratory (e.g., Simmons & Parker, 1992; Simmons et al., 1996; Ward & Simmons, 1991). However, after accounting for size-specific sperm transfer rates, we find that

sperm number significantly increases with male size on dung, and this pattern was sufficient to explain the positive association between male HTL and per mating reproductive success. On pomace, however, paternity share decreased with male body size, and this effect remained significant after controlling for focal male sperm expenditure. This finding could suggest that small males produce higher quality ejaculates or benefit from post-copulatory cryptic female processes that bias sperm usage in their favor. However, because this pattern is not observed for dung matings, a more plausible explanation may be that large males on pomace are transferring fewer sperm per unit time than expected due to depletion of sperm reserves. Distinguishing among these possible explanations will require detailed analysis of size-dependent sperm transfer rates and fertilization across environmental conditions and physiological status. Regardless of the proximate factor(s) driving patterns of sperm allocation and reproductive success, the results of our experiment demonstrate that small males, irrespective of mating environment, typically produce fewer offspring per copulation than large males on dung, and therefore, reinforce the assumption that alternative mating tactics in this species are conditionally employed by small males to “make the best of a bad situation.” However, additional work on size- and tactic-specific mating rates across different environmental contexts is needed to fully understand the maintenance and optimal expression of these alternative tactics.

Although there are many examples of small and large males adopting alternative ejaculation strategies when employing different behavioral tactics, few studies have analyzed how these males respond to changing social environments and/or mating roles. Results from available studies, however, are consistent with the size- and context-dependent patterns of ejaculate expenditure found in this study. For instance, in the European bitterling, *Rhodeus scericeus*, males also adopt size-dependent AMTs, and changes in population density (1) cause

males to flexibly switch between territorial and sneak tactics, (2) alter the relative fertilization success associated with each mating tactic and (3) drive dominant and subdominant males to differentially adjust their rate of ejaculation (Candolin & Reynolds, 2002; Reichard et al., 2004). Similarly, in the domestic fowl, *Gallus gallus domesticus*, experimental manipulation of social environment elicited a plastic reduction in the quality of sperm produced by socially dominant males, whereas sperm quality of socially subordinate males remained unchanged (Pizzari et al., 2007; Tazzyman et al., 2009). Our study provides further evidence that male expenditure is determined by complex relationships between male size, mating tactic and mating environment (i.e., mating role). Future work should address these relationships in other species that flexibly shift mating tactics and/or mating roles throughout life, with studies comparing ejaculate expenditure of individual males before and after switching tactics being of particular interest.

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Legends

Figure 1. Interaction plots showing relationship between male hind tibia length and (a) copula duration and (b) estimated number of sperm per ejaculate in both mating environments (grey = dung, black = pomace).

Figure 2. Proportion of a female's clutch sired per mating as a function of male hind tibia length for (a) pomace and (b) dung matings.

Table 1. Results of moderated multiple regression analyses, which tested the effects male hind tibia length (continuous), mating environment (dichotomous moderator) and their interaction on copula duration and total sperm number (estimated using known relationship between male size and sperm transfer rate). Temperature (not shown) was included as a covariate in all analyses.

Model	<i>N</i>	<i>R</i> ²	<i>F</i>	<i>β</i>	<i>SE</i> _{<i>β</i>}	<i>P</i>
<i>Copula duration</i>	130	0.4194	22.575			< 0.001
Male HTL				− 37.072	4.590	< 0.001
Env				+ 3.941	3.184	0.2181
Male HTL x Env				+ 39.049	9.279	< 0.001
<i>Sperm number</i>	130	0.3182	14.584			< 0.001
Male HTL				+ 595.406	164.320	<0.001
Env				+ 310.508	113.966	0.007
Male HTL x Env				+ 1667.681	332.168	< 0.001

595 **Table 2.** Statistics for binomial generalized linear models of paternity share on male body size (hind tibia length) for
596 matings on pomace or dung. Male 2 sperm number is incorporated in *Pomace* models to account for variation in
597 Male 1 paternity, while focal male sperm number is included in *Pomace (b)* and *Dung (b)* to assess whether
598 expenditure mediates size-dependent patterns of reproductive success.

Model	<i>N</i>	β	SE_{β}	<i>Z</i>	<i>P</i>
<i>Pomace (a)</i>	65				
Male 1 HTL		− 0.6588	0.26995	− 2.445	0.014
Male 2 sperm number		− 0.0005	0.0002	− 3.718	< 0.001
<i>Pomace (b)</i>	65				
Male 1 HTL		− 0.5841	0.2774	− 2.015	0.035
Male 1 sperm number		0.0004	0.0001	− 3.168	0.002
Male 2 sperm number		− 0.0005	0.0002	− 3.638	< 0.001
<i>Dung (a)</i>	65				
Male 2 HTL		1.015	0.2652	3.826	< 0.001
<i>Dung (b)</i>	65				
Male 2 HTL		0.4841	0.3441	1.407	0.159
Male 2 sperm number		0.0004	0.0002	2.620	0.009

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