

Original Article

Dominance status and carcass availability affect the outcome of sperm competition in burying beetles

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Intense intrasexual competition between males often results in the establishment of a dominance hierarchy, promoting differential access to females. Dominant males are expected to evolve more prudent sperm allocation strategies, whereas subordinate males are expected to counteract their mating disadvantage by increasing their sperm allocation. Burying beetles, insects that breed on small vertebrate carcasses, offer an ideal model for testing these predictions because aggressive interactions between males typically result in one dominant male that monopolizes access to the female, relegating subordinates to sneak matings. Males that have not found a carcass use pheromones to attract females and only obtain a small number of matings per day. We measured the mating frequency of males competing on a carcass and confirmed that dominant males mate significantly more often than subordinates. We then examined the paternity of competing males, with a 5:1 mating advantage for the dominant male, to determine whether relative mating frequency is sufficient to explain the severe reproductive skew in polyandrous broods in the field. When competing on a carcass, dominant males did not sire significantly more offspring than subordinates. However, when males mated without a carcass and did not interact, significantly more offspring were sired by the dominant male, but even here, the subordinate male's paternity was greater than his share of matings. Our results show that when males compete on a carcass, dominant males have a clear mating advantage, whereas subordinates appear to transfer greater numbers of sperm per mating to compensate for their limited mating opportunities. *Key words*: burying beetles, dominance, intrasexual competition, *Nicrophorus vespilloides*, paternity, sperm competition. [*Behav Ecol* 22:1079–1087 (2011)]

INTRODUCTION

Sperm competition occurs when the sperm of more than one male compete to fertilize a given set of ova (Parker 1970, 1998). Because of the costs of sperm production (Dewsbury 1982; Preston et al. 2001), models of sperm competition generally predict that males should adjust their sperm allocation in accordance with the reproductive success likely to be derived from each copulation and the probability of future mating success (Parker 1990a, 1990b; Reinhold et al. 2002). Empirical evidence has largely supported the prediction that males should optimize rather than maximize the number of sperm transferred at each mating (e.g., Gage 1991, 1995; Simmons et al. 1993; Schaus and Sakaluk 2001). Models typically assume that males optimize their allocation of a finite amount of sperm, thus disregarding sperm production and replacement during the mating period.

In some mating systems, intense intrasexual competition results in the establishment of dominance among males leading to differential access to females. Males in the dominant role are expected to evolve more prudent ejaculate strategies, adjusting ejaculate size in accordance with their higher probability of remating (Parker 1990a, 1990b; Ball and Parker 2000; Reinhold et al. 2002). For subordinate males, in contrast,

sneak copulations often represent the only way to obtain fertilizations in the presence of dominant males (Rudolfson et al. 2006), making mating opportunities both rare and unpredictable. Under these conditions, subordinate males are predicted to counteract the disadvantage of a lower mating frequency by increasing ejaculate size (Parker 1990a, 1990b, 1998). If mating also occurs in solitary contexts when males do not interact, fertilization success is predicted to deviate from that observed in social contexts (Shuster and Wade 2003).

The life history of burying beetles (genus *Nicrophorus*) makes them an ideal experimental model for the study of sperm competition in different mating contexts (reviewed by Eggert and Müller 1997; Scott 1998). Burying beetles are well known for the biparental care that males and females provide to their developing larvae, which they rear on small vertebrate carcasses. On finding a carcass, the beetles bury it, removing any fur or feathers, and gradually mold it into a ball-like shape during the process. After burying the carcass, the beetles continue to moisten and clean it until the larvae have completed their development. Throughout their development, the larvae are fed and defended by the adults.

Male *N. vespilloides* exhibit a plastic mating strategy involving 2 alternative tactics: 1) males without a carcass emit pheromones to attract females or 2) males fly in search of carcasses from which they advertise for a long-term breeding partner (Eggert 1992). In *N. vespilloides*, between 14% and 39% of broods have been found to involve lone females utilizing stored sperm from previous matings (Eggert 1992; Müller et al. 2007). Even when females bury a carcass with another

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male, some of the offspring result from eggs fertilized by previously stored sperm (Müller and Eggert 1989; Eggert 1992; Müller et al. 2007). Thus, sperm transferred by pheromone-emitting males likely always experience high levels of competition from other males' sperm.

When a carcass is discovered by more than one beetle, aggressive interactions between same-sex individuals typically result in one dominant pair that almost completely monopolizes access to the carcass (Pukowski 1933; Trumbo 1992; Eggert and Müller 1997). Larger males have a higher probability of winning aggressive interactions, and the victorious male subsequently defends the carcass and the female(s) from subordinate males. Although dominant males sire the majority of offspring on a carcass, they are usually unable to completely preclude matings by subordinates, and females do not reject the copulation attempts of vanquished males (Bartlett 1988; Dressel and Müller 1988; Müller et al. 1991; Eggert 1992). Hence, subordinate males often remain inconspicuous in the vicinity of the breeding pair, making occasional forays to the carcass to obtain matings with the resident female (Dressel and Müller 1988; Müller et al. 1991). In *N. vespilloides*, males that lose fights over a carcass have been shown to sire between 0% and 35% of the offspring (median = 10%) (Eggert 1992).

Matings thus occur in very different contexts. Pheromone-emitting males likely encounter 1 or 2 females per day if conditions are favorable (Eggert and Müller 1989) and can thus transfer a large proportion of their sperm in 1 or 2 matings. On carcasses, single males are known to mate an average of 79 times in 2 days (Müller and Eggert 1989), likely spreading their sperm out over a large number of matings. It appears reasonable to assume that dominant males obtain similar numbers of matings, whereas the mating frequency of subordinates probably falls somewhere in between dominant and pheromone-emitting males. Theoretically, therefore, we would predict that the largest ejaculates are transferred by pheromone-emitting males, followed by those of subordinate males on carcasses, with the smallest ejaculates transferred by dominant males or single males on carcasses.

Previous studies of *N. vespilloides* suggest that both male status and mating context play a role in mediating the outcome of sperm competition. In accordance with the predictions of sperm allocation models, Sakaluk and Müller (2008) found that subordinate males increased their copulation duration significantly in the presence of rival males, whereas dominant males did not alter their copulation duration in the presence of subordinates. The increased copulation duration of subordinate males did not, however, lead to an increase in paternity. House et al. (2007) compared the fertilization success of dominant and subordinate *N. vespilloides* males in different mating contexts. When rival males were each allowed to mate once with the same female without a carcass, their relative paternity varied, but the first and the second male to mate had roughly the same average fertilization success. However, when the same pairs of males were allowed to compete on a carcass for 24 h and to mate freely during that time, the larger of the 2 males sired the majority of offspring. It appears that when male rivals do not directly interact with each other but mate once with the same female in the absence of a carcass, fertilization success is determined by the competitiveness of the 2 males' ejaculates. When males interact directly with each other on carcasses, other mechanisms contribute to their relative fertilization success.

The most parsimonious explanation accounting for the success of larger males on a carcass is that dominant males are better able to secure matings with the resident female. The existing accounts of matings obtained by subordinate males do not provide numerical data, but state that subordinate matings are frequent (Dressel 1987; Bartlett 1988; Dressel and

Müller 1988; Müller et al. 1991). Müller and Eggert (1989) showed that high levels of paternity of parental males on carcasses are coincident with the increased mating frequency of parental males; however, the relative mating frequencies of dominant and subordinate males are unknown, and thus, we do not know if mating frequency and fertilization success are proportional.

Other alternative explanations accounting for the severe reproductive skew of dominant males cannot be discounted. For example, if female postcopulatory choice favors the sperm of dominant males (Eberhard 1996), this process could lead to a similar pattern (House et al. 2007). Furthermore, it is conceivable that only the dominant male can afford to produce and transfer larger amounts of sperm because of his unlimited access to a protein-rich food supply, the carcass. Regardless of the exact mechanism, intrasexual competition between male burying beetles apparently results in significant reproductive gains for dominant males.

The objectives of the present study were to 1) determine the mating success of males when competing on a carcass or when breeding alone with females and 2) test whether relative mating number is sufficient to explain the severe reproductive skew observed among males on carcasses. If repeated mating is a behavioral tactic employed by dominant males to outcompete their subordinate rivals in fertilizing the resident female's eggs, we predicted that 1) dominant males on carcasses would mate more frequently with the resident female than subordinate males and 2) dominant males would mate more frequently in the presence of a subordinate than when they were alone on a carcass with a female. If the relative number of matings a male obtains on a carcass is the prime determinant of his paternity, we predicted that 1) the fertilization success of dominant males would be proportional to their relative mating frequency and 2) if the relative number of matings was held constant across different mating contexts (i.e., with and without a carcass), the relative fertilization success of dominant and subordinate males would be unchanged. If, however, female preferentially use the sperm of dominant males when breeding on a carcass or if only dominant males can manufacture larger ejaculates by virtue of their greater access to a rich food source, then dominant males might be predicted to enjoy higher paternity in matings on carcasses than away from carcasses even if the relative number of matings is identical in both situations. If, instead, subordinate males allocate relatively greater amounts of sperm to compensate for their decreased mating success when mating on a carcass, then we might predict that dominant males would have lower paternity in matings on carcasses than away from carcasses.

MATERIALS AND METHODS

Experiment 1: mating frequency of males on a carcass with or without a competitor

Mating frequency of males on a carcass with and without a competitor was determined using first-generation *N. vespilloides* offspring of beetles trapped in a deciduous forest near Freiburg, Germany. Experimental males and females were housed individually on eclosion and fed raw beef twice a week for 3 weeks to ensure their sexual maturity. At the time of the experiments, all experimental beetles were virgin and 20–60 days old. All beetles were maintained at 20 °C under a 16:8 h light:dark regime. Carcasses used in the experiment were between 20 and 25 g.

Video recordings were made of the mating behavior of male *N. vespilloides* in 1 of 2 social contexts, one in which a single male was placed on a carcass with a female ($N = 10$), and the

other in which 2 competing males were placed on a carcass with a female ($N = 10$). Each trial was staged under dim red light and recorded over a 24-h period using a Panasonic CCTV camera (WV-BP330) equipped with a varifocal lens (Computar TG4Z2813FCS) and connected to a Panasonic time-lapse video recorder (AG-RT650). After measuring their pronotum width to the nearest 0.01 mm using digital calipers, experimental beetles were introduced simultaneously into a plexiglass arena (20×20 cm) containing a 1-cm layer of moist peat and provided with a mouse carcass. In such a situation, dominance is easily determined by observing encounters between males (Sakaluk and Müller 2008). Males in pairs were distinguished in video recordings by marking one of them on the pronotum with a white paint marker. On review of the video recordings, the number and duration of copulations were recorded. The mean ratio of copulations by the dominant and subordinate males when competing on a carcass was used to establish appropriate mating frequencies for the second experiment.

Experiment 2: reproductive success of males mated on and off a carcass

Two color strains of burying beetles (light and dark elytra) of *N. vespilloides* were used to determine the fertilization success of dominant and subordinate males on and off a carcass. The strains were developed through selective breeding of individuals originally caught in Freiburg, Germany and reared under standardized laboratory conditions (e.g., Müller 1987; Müller and Eggert 1989). Using these strains, the paternity of offspring whose potential sires are from different strains is easily assessed by evaluating the phenotype of adult offspring (Müller and Eggert 1989; Eggert and Müller 1992; Sakaluk et al. 1998). Experimental males and females were housed individually on eclosion and fed mealworms twice a week for 3 weeks to ensure their sexual maturity. As in the previous experiment, all experimental beetles were virgin and 20–60 days old. All beetles and subsequent offspring were maintained at 20 °C under a 16:8 h light:dark regime. Experimental matings took place within 3 h before lights went out, the period of time during which the beetles are most active (Eggert 1992). Prior to mating trials, the pronotum size of all males was recorded using an ocular micrometer mounted on a stereomicroscope.

Pairs of virgin male *N. vespilloides* were established in which the males in each pair came from different color strains and differed in pronotum width by at least 0.2 mm to ensure that the outcome of dominance interactions was predictable (Sakaluk and Müller 2008). These males were allowed a prescribed number of matings with each of 2 full-sibling females (unrelated to either male) in 2 mating contexts: while competing on a carcass and in the absence of a carcass. In each context, the larger (presumably dominant) male was mated to the female 5 times in succession, followed by a single mating by the smaller (presumably subordinate) male. The 5:1 ratio of matings for dominant and subordinate males was within the range of the ratio of dominant:subordinate matings observed in video observations while maintaining an experimentally feasible number of matings for a study requiring direct observation.

The color strains of the males and females, and the order in which males experienced the 2 carcass treatments, were alternated across every other replicate to control for any potential effects of color strain or treatment order on paternity. Males were returned to their individual containers to recover for a period of 48 h between treatments to ensure that they had sufficient time to replenish their sperm stores. When males experienced the “no carcass” treatment first, the larger male

was presumed to be the dominant, as size is a reliable indicator of dominance in *N. vespilloides* (Otronen 1988; Müller et al. 1990). Dominance was subsequently verified in the treatment in which males were allowed to compete directly for a carcass. Because males that arrive on a carcass are typically sexually experienced and to allow for the purging of older infertile sperm, all males were allowed to mate with an unrelated female 24 h prior to the start of their first mating trial. In both treatments, copulation durations were recorded.

In the treatment in which males competed for a carcass, both males and a virgin female were simultaneously released into a plexiglass arena (20×20 cm) containing 1 cm of moist peat and a thawed mouse carcass (24.56 ± 0.28 g). Direct observation ensured that dominance was established prior to any copulation and that the dominant male mated the prescribed number of times, after which he was removed from the arena. The subordinate male was then allowed a single mating with the female prior to being removed. Any mating attempts by the subordinate prior to his scheduled mating were carefully thwarted by the investigator with the use of forceps. The female and the carcass were then transferred to a smaller container with peat where she was allowed to continue to prepare the carcass and lay eggs.

In the absence of a carcass, the same males were allowed the same number of matings in the same order, this time with a virgin sister of the female used on the carcass. Experimental beetles were held in individual transparent plastic cups (10 cm diameter \times 10 cm height) lined with a sheet of moistened paper towel. Matings took place at 20-min intervals by placing the male inside the container of the female, and after a copulation had occurred, the male was returned to his own holding container. The dominant male was allowed the first 5 matings, and subsequently the subordinate male was allowed to mate once. Immediately after the last mating, the female was placed in a container containing 3 cm of moist peat and a thawed mouse carcass as in the previous treatment and allowed to prepare the carcass and lay eggs. In both treatments, containers with females on carcasses were maintained in an incubator in complete darkness up on the burial of the carcass.

Females in both treatments were allowed to rear offspring as they would in a natural breeding attempt. Broods were checked daily to ascertain when parental care was complete. At the end of larval development when larvae disperse from the remains of the carcass, larvae were removed and placed into new containers filled with moist peat in groups no larger than 10 to undergo pupation. Pupation boxes were kept in complete darkness at 20 °C. Offspring emerging as adults were counted, and paternity was assessed based on elytral coloration.

We established a total of 34 experimental male pairings resulting in 68 mated females. In 3 cases, it turned out that the smaller of the 2 males was the dominant; these pairs were eliminated from further analysis because the mating frequency of the dominant and subordinate males in these 3 pairs would have been the reverse of the others.

Statistical analyses

All statistical tests were performed using SAS version 9.1 (SAS Institute 2004), and all means are given ± 1 standard error. The number of copulations by dominant and subordinate males competing on the same carcass was compared using a paired *t*-test. The number of copulations by dominant males with and without a competitor was analyzed using a repeated-measures analysis of variance (ANOVA), with time interval 6-h blocks) as the within-male factor and the presence or absence of a subordinate as the between-subjects factor.

To compare the copulation durations and number of offspring sired by each male across mating contexts in the second

experiment, we employed a repeated-measures ANOVA in PROC MIXED, with male dominance and carcass treatment as within-pair factors. Pairwise comparisons of significant effects were evaluated using the LSMEANS option.

RESULTS

Experiment 1: mating frequency of males on a carcass with or without a competitor

One male died before the end of the 24-h recording period in each of the 2 treatments, leaving a total of 18 replicates for further analysis (9 in each treatment). In trials involving 2 competitors, dominance was always clearly established after an initial period of fighting, with one male (the dominant) retaining full control of the carcass and repelling the other male (the subordinate) in any subsequent encounter. Dominant males obtained 59–89% of all copulations and copulated significantly more often during the 24-h recording period (100.2 ± 14.2) than subordinate males (39.0 ± 8.53 ; paired *t*-test, $t_8 = 4.96$, $P = 0.0011$). There was no significant correlation between male body size and copulation frequency for either dominant males (Spearman rank correlation; $N = 9$, $r = -0.17$, $P = 0.67$) or subordinate males (Spearman rank correlation; $N = 9$, $r = 0.06$, $P = 0.88$). There was, however, a significant correlation between the number of copulations by subordinate males and dominant males (Spearman rank correlation; $N = 9$, $r = 0.72$, $P = 0.0298$) (Figure 1).

Over the same time period, solitary males without a competitor copulated 83.1 ± 7.8 times; there was no significant correlation between the body size of solitary males and their mating frequency (Spearman rank correlation; $N = 9$, $r = -0.10$, $P = 0.80$). There was no significant difference in the copulation frequency of solitary males and dominant males mating in the presence of a subordinate ($F_{1,16} = 1.12$, $P = 0.3057$). The copulation frequency of males in both situations varied significantly over time ($F_{3,48} = 12.25$, $P < 0.0001$), with a higher frequency of mating occurring in the first 12 h than in the last 12 h of the recording period (Table 1, Figure 2). There was no significant interaction between time and treatment in their effects on male copulation frequency ($F_{3,48} = 1.49$, $P = 0.229$).

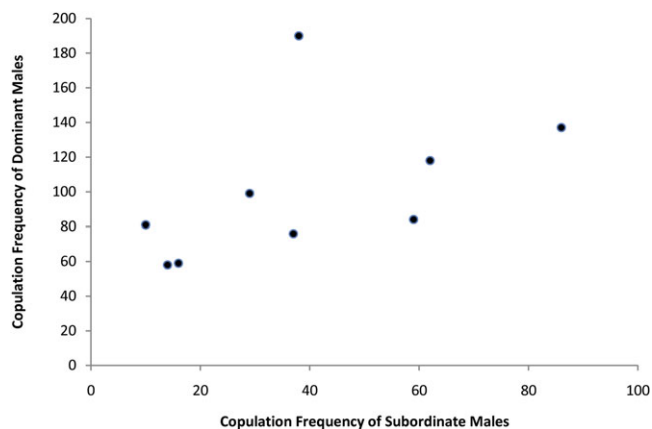


Figure 1

Scatter plot of the number of copulations in a 24-h period by dominant and subordinate males within pairs of competing males. The correlation between the 2 variables was significant (Spearman $r = 0.72$, $P = 0.0298$).

Experiment 2: reproductive success of males mated on and off a carcass

We analyzed the effect of male status (dominant and subordinate) and carcass availability (with and without a carcass) on male reproductive success using each pair of males as the experimental unit. Because male status was repeated across both levels of carcass availability, we analyzed the effects of male status and carcass availability as within-subject repeated factors.

Copulation durations of dominant and subordinate males

We could not accurately determine the mating duration of one male, and so this individual was excluded from the analysis of copulation duration.

There was a significant interaction between male status and carcass availability in their effect on copulation duration (Table 2; Figure 3). Pairwise comparisons using least-squares means revealed that both dominant males (using the average of 5 copulations with the same female, $t_{54.3} = 10.84$, $P < 0.0001$) and subordinate males ($t_{54.3} = 5.94$, $p < 0.0001$) had significantly longer copulations when mating in the absence of a carcass than when mating on a carcass. There was no significant difference in the copulation durations of dominant and subordinate males when mating on a carcass ($t = 0.79_{57.5}$, $P = 0.4304$), but dominant males copulated significantly longer than subordinate males when mating in the absence of a carcass ($t_{57.5} = 4.61$, $P < 0.0001$).

Number of offspring sired by dominant and subordinate males

Of the 62 mated females, 12 failed to lay eggs or laid infertile eggs, leaving 24 females mated on a carcass, and 26 mated off a carcass to be included in the paternity analysis. Because of the unbalanced nature of the data, parameter estimates were obtained in PROC MIXED (SAS Institute 2004) using REML, and degrees of freedom were estimated using the Satterthwaite approximation. The mean brood size of females mated on a carcass (13.6 ± 2.3) did not differ significantly from that of females mated in the absence of a carcass (19.0 ± 2.6) (Paired *t*-test, $t_{23} = 1.81$, $P = 0.0836$).

There was a significant interaction between male status and carcass availability in their effect on the number of offspring sired (Table 3; Figures 4 and 5). Pairwise comparisons using least-squares means revealed that dominant males sired more offspring than subordinate males when mated in the absence of a carcass ($t_{75.1} = 3.08$, $P = 0.0029$), but there was no

Table 1

Repeated-measures ANOVA examining the effect of the presence of a rival male and time on the number of copulations

Source	Type III			
	df	Mean Square	<i>F</i>	<i>P</i>
Between subjects				
Treatment ^a	1	329.39	1.12	0.3057
Error	16	294.23		
Within subject				
Time ^b	3	629.28	12.25	<0.0001
Time × treatment ^b	3	76.537	1.49	0.229
Error(time)	48	51.355		

^a The *F* value for the effect of treatment was calculated using the mean squares error between treatment groups (df = 16).

^b The *F* value for the effects of time and the interaction between time and treatment was calculated using the within-time mean squares error (error(time); df = 48). df, degrees of freedom.

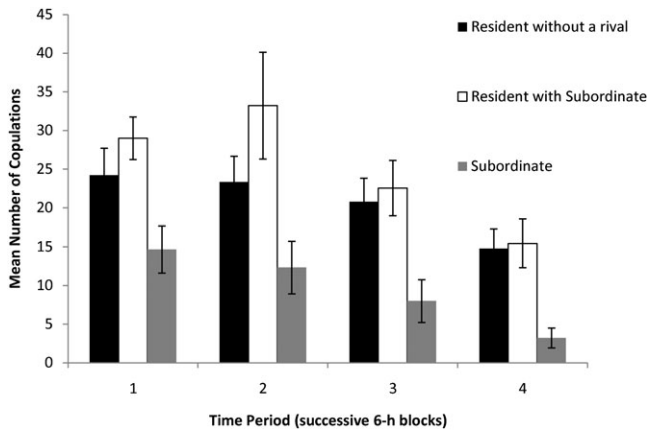


Figure 2
Mean (\pm standard error) number of copulations obtained by solitary males (white bars), dominant males competing against a subordinate (black bars), and subordinate males (gray bars).

significant difference in the number of offspring sired by dominant and subordinate males when mating on a carcass ($t_{75,8} = 0.49$, $P = 0.6275$). Dominant males sired significantly more offspring when mating in the absence of a carcass than when mating on a carcass ($t_{43,3} = 2.56$, $P = 0.0139$), but carcass availability had no significant effect on the number of offspring sired by subordinate males ($t_{75,1} = 3.08$, $P = 0.6683$).

To determine whether the paternity achieved by dominant and subordinate males was directly proportional to the number of times they were mated to experimental females, we employed a binomial test with the null expectation that dominant males should sire 5/6 (83.3%) of the offspring based on their 5 matings to the subordinates' one. Dominant males sired proportionally fewer offspring than we would expect based on their relative mating frequency (Figure 5), both when mated on a carcass (154 of the 278 offspring [55%], $P < 0.0001$) and when mated in the absence of a carcass (299 of the 409 offspring [73.1%], $P < 0.0001$). Furthermore, the proportion of offspring sired by dominant males was significantly different when mated in the absence of a carcass than when mated on a carcass ($\chi^2 = 23.11$, $P < 0.0001$).

DISCUSSION

The results of our video study confirm anecdotal reports that subordinate matings are frequent (Dressel 1987; Bartlett 1988; Dressel and Müller 1988), and they document for the first time that dominant males obtain significantly more matings than subordinate males. The number of subordinate matings may even have been somewhat inflated by our experimental setup, which effectively reduced the beetles' 3-dimensional

Table 2
Repeated-measures ANOVA model examining the effect of male status (dominant or subordinate) and carcass availability on copulation duration

Source	Error			
	df	df	F	P
Copulation duration				
Male status	1	29	6.63	0.0154
Carcass availability	1	29	111.76	<0.0001
Status \times carcass availability	1	29	16.17	0.0004

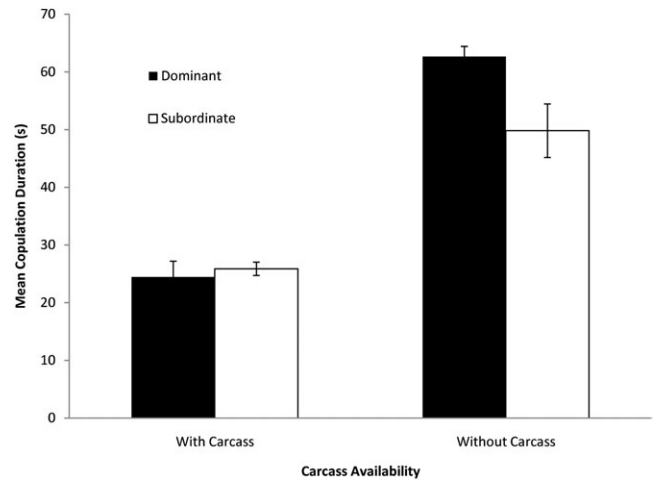


Figure 3
Mean (\pm standard error) copulation durations of dominant and subordinate males mating on a carcass or in the absence of a carcass.

environment to 2 dimensions. The observation chamber was far less structured than a stretch of forest floor in the field and thus offered fewer opportunities for the subordinate to hide and avoid encounters. The female frequently moved along the edges of the observation chamber, which increased her encounter rate with the hiding subordinate and afforded him more opportunities to mate than he might have had otherwise (Pettinger AM, personal observations). Even under these conditions, however, subordinates obtained significantly fewer matings than dominant males.

Compared with males that were alone with a female on a carcass, dominant males competing with a subordinate male did not mate significantly more often in their first 24 h on the carcass. This result suggests that the presence of a subordinate male did not affect the resident male's mating frequency, contrary to our original prediction. However, our sample size was small, and there was considerable interindividual variation between males, and under such conditions, a paired design might have been more powerful in helping us detect potential differences. Another observation, however, suggests that dominant males may respond to the presence of a subordinate after all: The mating frequency of the dominant male was positively correlated with the mating frequency of the subordinate rival, suggesting that dominant males adjust their copulation frequency in accordance with the risk of sperm competition. Dominant males may have assessed the threat to their reproductive success posed by a competitor based on their frequency of encounters with subordinates or the time the female was absent from the carcass. The correlation

Table 3
Repeated-measures ANOVA model examining the effect of male status (dominant or subordinate) and carcass availability on the number of offspring sired

Source	Error			
	df	df ^a	F	P
Offspring sired				
Male status	1	47.3	4.90	0.0316
Carcass availability	1	43.3	2.27	0.1390
Status \times carcass availability	1	43.3	4.48	0.0400

^a Exclusion of some data resulted in an unbalanced design in analyzing offspring sired. Therefore, we employed REML and Satterthwaite degrees of freedom (df).

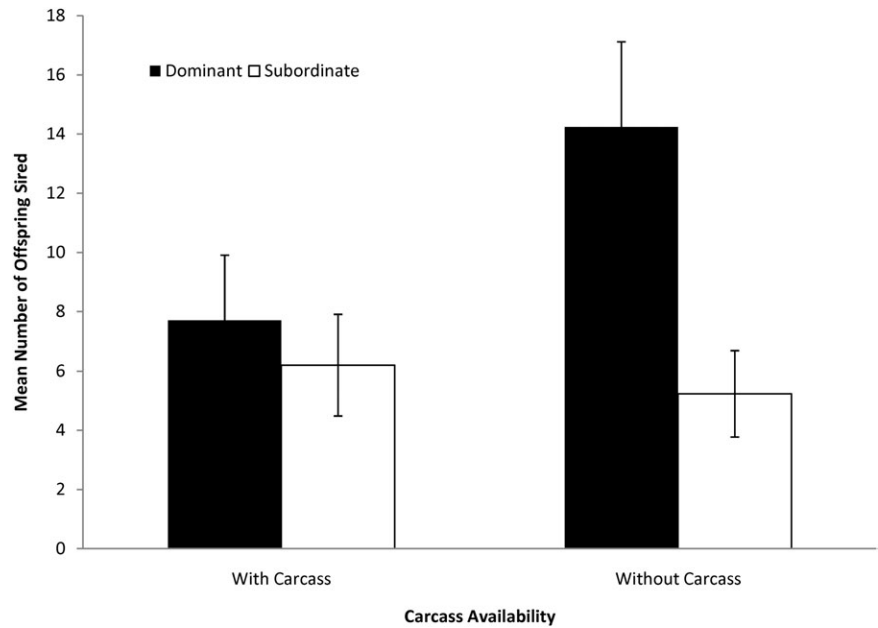


Figure 4
Mean number (\pm standard error) of offspring sired by dominant and subordinate males when mating on a carcass or mating in the absence of a carcass.

warrants further study, however, as we cannot exclude the possibility that it was merely some uncontrolled confounding variable that caused the mating frequency of both males to increase in some trials (e.g., variation in female attractiveness or receptivity).

We were also able to document changes in mating frequency over the course of males' first 24 h on a carcass. Both solitary males and dominant males mating in the presence of a subordinate mated more frequently in the first 12 h than in the last 12 h of the recording period. However, there was no significant interaction between time and treatment in their effects on male copulation frequency, suggesting that the presence of a subordinate did not affect the temporal pattern of mating of resident males. The mating frequency of subordinate males, in contrast, decreased steadily over the course of the first four 6-h intervals. It is conceivable that resident males, with or without a subordinate rival, exhibit their greatest mating effort during the first 12 h after the discovery of a carcass to achieve maximum overlap with the female's oviposition period. How-

ever, Smiseth et al. (2006) found that females lay the majority of their eggs between 12 and 24 h after finding a carcass, and thus, the highest rates of mating occur well before egg-laying commences. This suggests that either sperm have a greater chance of being used if transferred earlier or that females are more reluctant to mate during the actual period of oviposition. Female rejection behavior during oviposition might benefit not only the female but also the dominant male, as females oviposit in the substrate away from the carcass and are thus more likely to encounter subordinate males hidden in the soil. The dominant male remains on the carcass, and females returning there may be more willing to mate than when they are ovipositing. The continuous decline in the mating frequency of subordinate males was less likely a function of strategic sperm allocation than a function of constraints imposed by the dominant limiting the subordinate male's ability to approach the carcass and the female without being killed or injured. It may be that over time, the dominant becomes more efficient at controlling access to the carcass,

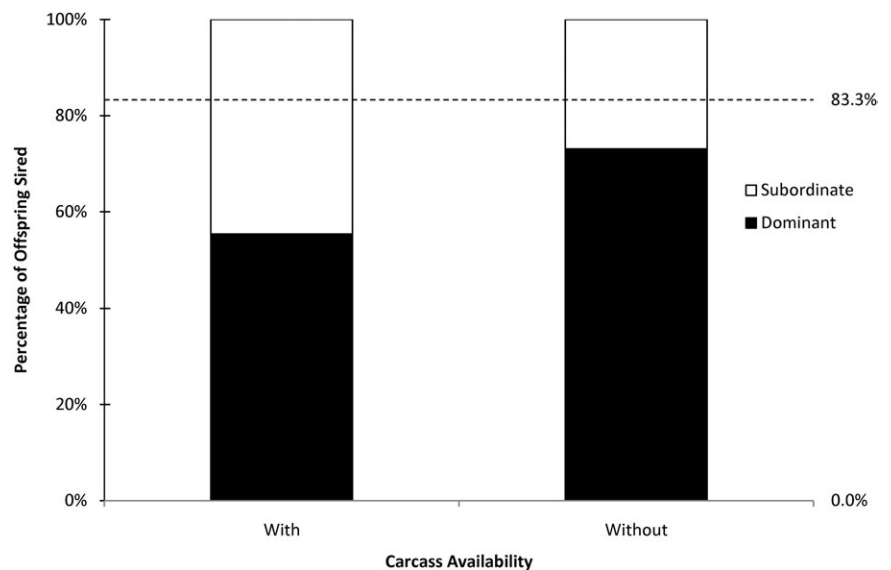


Figure 5
Percent of all offspring sired by dominant and subordinate males when mating on a carcass or mating in the absence of a carcass. Dashed line indicates percent paternity expected based on relative number of matings.

a task that may become easier once interment has been completed. It is conceivable that the subordinate increasingly exhausts his sperm reserves such that matings become less profitable for him over time. Female egg production is limited by access to food (Eggert et al. 2008), and if sperm production is subject to similar constraints, a lack of feeding opportunities may cause the subordinate to deplete his supply of fertile sperm faster, or replenish it more slowly, than the dominant.

There was a significant interaction between male dominance status and carcass availability, both in their effect on copulation duration and on the number of offspring sired. When no carcass was present, dominant males mated longer than subordinates, but on carcasses, they did not. Copulations away from a carcass were more than twice as long as copulations with a carcass, regardless of male status. Although we know nothing about the actual transfer of sperm with varying copulation duration, it is reasonable to assume that longer copulations result in the transfer of greater numbers of sperm, and thus, are indicative of greater male investment in ejaculates during matings away from carcasses. In the field, matings without carcasses only occur when males emit pheromone; in this situation, males only mate once with each female they attract (Eggert and Müller 1989). Sperm transferred in such matings nearly always have to compete with other males' ejaculates for fertilizations (Müller and Eggert 1989) because it is uncertain when an inseminated female might find a carcass and what the breeding association there might be. In such a situation, males are predicted to exhibit much less prudent sperm allocation than on carcasses, where mating frequency is high and sperm competition lower (Parker 1990a, 1990b). Moreover, the expected mating rate for pheromone-emitting males is very low. Field studies of males emitting pheromone without a carcass have revealed that males employing this tactic attract an average of 1 female per day over all days they spend pheromone emitting (Eggert and Müller 1989). Because each of these females allows only a single copulation, the average mating rate for a pheromone-emitting male is only 1 per day.

However, other factors may contribute to the difference in the duration of matings on and off a carcass. For dominant males, shorter copulations on a carcass may be imposed by the need to conceal the carcass quickly, and their continued unlimited access to females throughout the breeding attempt may enable them to opt for more frequent short matings rather than fewer long ones. Subordinate males might be forced to keep copulations brief around carcasses because longer matings increase their risk of being caught in the act of copulation by the dominant. A subordinate that is attacked while mating may have little opportunity to avoid an escalated aggressive encounter. After the initial fights that establish dominance, subordinates usually flee as soon as they perceive an approaching dominant, and mating could interfere with this rapid response.

In interactions on carcasses, subordinates were predicted to exhibit greater sperm allocation per copulation than dominants because of their mating disadvantage. Indeed, studies of other taxa have provided some support to this prediction (e.g., Stockley et al. 1994; Rudolfson et al. 2006; but see Simmons et al. 2000). The similar mating duration for both males on carcasses in the present study did not support this prediction, although the mean copulation duration of subordinate males was slightly, albeit nonsignificantly, greater. Sakaluk and Müller (2008) found that subordinate males mated significantly longer than dominant males in the presence of a carcass. Their study likely had a lower measurement error than ours, as they observed pairs in small transparent observation containers offering high visibility. When we observed beetles on carcasses, pairs often crawled under the carcass or into the peat layer while mating, interfering with our ability to precisely measure the period during which the

genitalia were coupled. This less-than-perfect visibility probably increased our measurement error from less than 0.5 s in the transparent containers to 2–3 s on the carcass, a significant change given that the difference in copulation duration found by Sakaluk and Müller (2008) was only 4 s.

We also found that matings of dominant males without a carcass were significantly longer than those of their subordinate rivals. This result is surprising given that in the absence of a carcass, both males were in the same situation as a male emitting pheromone (mating once with a female that might at some later time find a carcass), and thus, they were expected to exhibit similar sperm allocation patterns. We offer 2 possible explanations for this result based on the 2 systematic differences between males in the experiment: By design, dominant males were slightly larger than their assigned subordinates, and they were always first to mate. Larger males might produce larger ejaculates, necessitating longer copulation durations. If males are capable of assessing the mating status of prospective mates, mating order may have mattered: Dominant males encountered a female that was virgin during their first mating, and then, a partner with whom they themselves had mated previously, whereas subordinate males encountered a female that had already been mated 5 times by another male, and thus, might have appeared less valuable. This might have caused subordinates to decrease their sperm allocation through reduced copulation duration. Although the same logic could in principle apply to matings on carcasses, the perceived value of the female there is likely to be very different as she is about to produce and fertilize eggs on the carcass.

Female behavior also could have influenced the duration of copulations both on and off carcasses. Often, females remain motionless during copulation, but based on anecdotal observations, female movement may have delayed or curtailed matings. In matings away from carcasses, it appeared that it was usually the male rather than the female that terminated the mating (Pettinger AM, personal observations). On carcasses, female activity could conceivably have a greater effect because males may become dislodged when a female crawls underneath the carcass (Pettinger AM, personal observations). Thus, it is possible that female behavior during copulation could represent a form of cryptic female choice (Crudgington and Siva-Jothy 2000), but currently there is no direct evidence that female behavior influences copulation duration or paternity in *N. vespilloides*. In addition, matings in Sakaluk and Müller's (2008) study were similarly short as in ours, despite the fact that for observation of matings, males were removed from the carcass and pairs observed in small containers that offered no more opportunity for female interference than the containers we used to observe pairs away from carcasses.

The interaction between male dominance status and carcass availability in their effect on copulation duration presaged a similar interaction with respect to male fertilization success: Dominant males sired more offspring than subordinate males when mated in the absence of a carcass, but there was no significant difference in the number of offspring sired by dominant and subordinate males when mating on a carcass. This suggests that differences in copulation duration mediate, to some extent, the outcome of sperm competition. Although we do not know the exact relationship between copulation time and number of sperm transferred, it is reasonable to assume that this relationship is positive, with the transfer rate diminishing after a while, as has been documented in other insects. If this is the case, it is also reasonable to expect that males transfer more sperm away from a carcass, where matings are twice as long as on carcasses.

Unexpectedly, we also detected a possible effect of carcass availability on the total number of offspring sired: Females

mated without a carcass produced more offspring than those mated on a carcass, but the difference was not quite statistically significant. This difference is, nevertheless, suggestive and could be a consequence of the significantly shorter times females on carcasses spent in copulation. Although in many insects, a single copulation is sufficient to fertilize a female's lifetime production of eggs (Parker 1970), in female *N. vespilloides* that had been mated once, about half of the eggs laid by females remained unfertilized (House et al. 2008, 2009). These single matings had been staged in the absence of a carcass and thus should have been relatively long but were apparently still not sufficient to fertilize an entire clutch of eggs, and even after 12 matings, fertilization rates were still under 80% (House et al. 2008).

Dominant males sired a significantly greater proportion of offspring than subordinate males when given a 5:1 mating advantage but only when matings occurred in the absence of a carcass. If the number of matings alone was responsible for the much higher paternity of dominant males on a carcass observed in the natural situation (Eggert and Müller 1989; House et al. 2007, 2008; Müller et al. 2007), then paternity should have been directly proportional to the relative number of matings, regardless of mating context, which was not the case. Although similar copulation durations for dominant and subordinate males on a carcass suggest a similar sperm allocation per copulation, a 5:1 mating ratio did not yield a concomitant skew among offspring sired for the majority of pairs. The subordinate achieved far greater paternity than predicted based on his share of all matings (see Figure 5). Based on our results, there is no indication that females preferentially use the sperm of dominant males. If they did, the effect was completely overshadowed by some opposing influence that favored the subordinate male's sperm. There are a number of potential explanations for the greater fertilization efficiency of subordinate ejaculates. First, we expect that subordinate males should exhibit greater sperm allocation per mating than dominants only on carcasses, where subordinates have limited mating opportunities, similar to patterns found in dung beetles (Tomkins and Simmons 2000). In addition, our experimental design was such that subordinate males were always the last to mate. Thus, if the sperm transferred last prior to oviposition have an advantage in fertilizing eggs, this would have favored the subordinate male in each case. Last-male sperm precedence occurs in a variety of insect species and may be due to physical displacement or removal of sperm. House et al. (2007) found no systematic pattern of precedence in females that had mated twice with different males, but last-male precedence may only be apparent when female sperm storage organs are largely filled, which is not the case after 2 matings (House et al. 2008). Müller and Eggert's (1989) results strongly suggest the occurrence of last-male precedence in *N. vespilloides*: Females who mated about 50 times with a first male and only 7 times with a second male, produced young that were equally likely to be the first or the second mate's offspring.

Our results are consistent with the general prediction that males in the subordinate role should make a greater sperm allocation at mating (Parker 1990a). However, if the mating ratio we observed in our video study is reflective of the field situation, why were the patterns of sperm precedence documented in our paternity experiment not more similar to reproductive attempts in the field, where dominant males typically sire more than 90% of the offspring (Müller and Eggert 1989)? We offer 5 hypotheses that could account for the discordance between the laboratory and field results:

1) Confining the subordinate male may have artificially increased his mating success compared with that occurring in the field, and therefore, the ratio of matings observed in our video study may have overestimated the subordinate

male's share; 2) Dominant males may choose not to invest as heavily in sperm allocation during the initial time period, choosing instead to invest in burying the carcass and limiting the number of competitors; and 3) The timing of matings may have important effects on male fertilization success because of the positioning of sperm inside the female's reproductive tract. In a natural situation, dominant males may optimize the timing of matings, increasing mating frequency when the female is closer to egg laying. Our results from the video study appear to support this hypothesis, as dominant males increased their mating frequency and maintained a high rate throughout the 24-h observation period. In our paternity study, however, dominant males were allowed only a few matings shortly after being provided with a carcass, and the sperm transferred in these matings were the only ones available for fertilizations; 4) Because there is last-male precedence, our experimental design likely overestimated the subordinate's expected paternity; and 5) Dominant male sperm production may increase with male residency on a carcass, whereas subordinate sperm production may decline due to their differential access to food or possibly due to hormonal processes akin to the ones that trigger vitellogenesis, oocyte development, and oviposition in females (Trumbo and Robinson 2004).

Our results must be viewed in light of what is known about the natural history of burying beetle breeding associations. The optimal sperm allocation strategy for a dominant, subordinate, or pheromone-emitting male is likely to be shaped by a set of costs and benefits unique to each of these mating tactics. Ejaculates from dominant males likely face competition from both stored sperm and subordinate ejaculates. However, dominant males have access to a virtually unlimited supply of nutrients and unfettered access to females. With unrestricted access to females, it is probably less important for males to transfer a large number of sperm at each mating, but rather, a strategic temporal distribution of matings becomes vital. Subordinate males, in contrast, face restricted access to females, high levels of sperm competition from dominant males, and potential injuries inflicted by the dominant males should they be discovered during attempted copulations. In such a scenario, males should be selected to maximize sperm transfer at each mating. Our results suggest that subordinate males do indeed transfer more sperm per mating than dominant males when mating on a carcass. Lastly, sperm from pheromone-emitting males are likely to be stored for an unknown period of time and forced to compete against an unknown number of ejaculates from other males. The value derived from such copulations is likely to be highly variable. Here, we found that males appeared to invest more heavily in ejaculates away from a carcass, as predicted by models assuming a fair raffle (Parker 1990a). Overall, our results are congruent with the hypothesis that males strategically adjust mating behavior and sperm allocation to the different mating contexts they face.

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