

Extreme variability in sperm precedence in the fungus beetle, *Bolitotherus cornutus* (Coleoptera Tenebrionidae)

JEFFREY K. CONNER¹

Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA
and

Department of Ecology, Ethology, and Evolution, University of Illinois, Urbana, IL
61801, USA

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Most previous studies of sperm precedence have reported only average values for species. Intraspecific variation in sperm precedence, however, is necessary for the evolution of adaptations that alter sperm precedence patterns. To examine intraspecific variation in sperm precedence, virgin female fungus beetles were mated to two different males in succession, and all females, males, and offspring were genotyped at eight allozyme loci. The proportion of offspring fathered by the second male varied from zero to one. Future studies should not only measure intraspecific variability in sperm precedence, but should attempt to determine causes of this variability.

KEY WORDS: sperm precedence, sperm competition, fungus beetle, *Bolitotherus cornutus*.

INTRODUCTION

Sperm competition can be an important determinant of male fitness in species with female multiple mating and sperm storage (PARKER 1970, SMITH 1984). In the past, most studies of sperm competition have focused on determining a species-typical pattern of sperm precedence, and so have reported average precedence values (LEWIS & AUSTAD 1990, but see DICKINSON 1986, MASON & PASHLEY 1991, SIMMONS & PARKER 1992). Intraspecific variation in sperm precedence, however, is necessary for the evolution of sperm precedence or adaptations affecting sperm precedence. Intraspecific variation in sperm precedence was found in *Tribolium* flour

¹ The author can be contacted at: Department of Ecology, Ethology, and Evolution, University of Illinois, Shelford Vivarium, 606 E. Healey St., Champaign, IL 61820, USA.

beetles by LEWIS & AUSTAD (1990); they also reviewed other studies that reported intraspecific variability. In this note I report extreme variability in sperm precedence in another species, *Bolitotherus cornutus*, a tenebrionid fungus beetle.

MATERIALS AND METHODS

For details of the biology of *B. cornutus* see LILES (1956), PACE (1967), BROWN & SIEGFRIED (1983), and CONNER (1988, 1989). To obtain virgin female *B. cornutus*, larvae were collected from fungi in February and May 1986 and raised in the laboratory. After the females matured, they were housed in individual plastic boxes. Matings were conducted from 21 May to 28 August 1986, which is the natural mating season of *B. cornutus* (CONNER 1988). Field-collected males were used to maximize the relevance of the results to natural populations, and because lab-reared beetles are usually smaller and less vigorous than field-collected individuals (CONNER unpub.). Males were introduced into the females' boxes, and all boxes were checked for post-copulatory guarding behaviour at least once every 2 hr. Guarding is a reliable indicator that spermatophore transfer has occurred, and typically lasts at least 2 hr (CONNER 1989). When guarding behaviour was observed, the male was removed and a new male from a different site was introduced. Males from different sites were used to maximize the possibility of electrophoretic differences between them; the order in which males from different sites were presented was randomized. After a female accepted sperm from two different males, she was left in her box to oviposit and the fungus in the box was changed weekly. All eggs found when the fungus was changed were placed in individual petri dishes for rearing on fresh-frozen slices of *G. applanatum*. Eggs were laid from June through September.

In all, 54 females accepted sperm from two males, but only 23 laid eggs and in only 13 of these families were four or more offspring successfully raised to late instar larvae or adults for electrophoresis. These 13 families were used to study sperm precedence. It is possible that using only 13 out of 54 families could bias the sperm precedence results, but it seems likely that the 13 families were a random sample with respect to sperm precedence patterns. The low fecundity of the females was likely due to the females being raised from larvae in the laboratory, in which the fungal resource is not living. The 13 females laid a total of 276 eggs, of which 51 (18%) died before they were large enough for electrophoresis; another 37 were not successfully genotyped for a total of 188 offspring used in the analyses.

Starch-gel electrophoresis was performed on parents and offspring using methods described by MAY et al. (1979). Eight enzyme loci were used: alcohol dehydrogenase (ADH, 2 alleles), esterase (EST, 2 alleles), glucosephosphate isomerase (GPI, 2 alleles), hydroxybutyric dehydrogenase (HBDH, 3 alleles), isocitrate dehydrogenase (IDH, 2 alleles), peptidase with phenyl-alanyl-proline (PEP, 4 alleles), phosphogluconate dehydrogenase (PGD, 3 alleles), and triose phosphate isomerase (TPI, 3 alleles). In seven of the families the electromorphs of some of the offspring were ambiguous, i.e., they could have been fathered by either sire. In these cases, the proportion of offspring sired by each male was estimated using a maximum likelihood function (DICKINSON 1986, DICKINSON & McCULLOCH 1989).

RESULTS AND DISCUSSION

The electrophoretic paternity analysis showed that sperm precedence values varied from complete first male precedence to complete last male precedence (Table 1). The average proportion fathered by the second male for all 13 families was 0.67, which was not significantly different from 0.50 by a one-sample t-test ($P = 0.10$). This mean is similar to the average of 0.62 reported for another tenebrion-

Table 1.

Sperm precedence in *Bolitotherus cornutus*.

Family	Proportion fathered by 2nd male	Loci used	# of offspring scored
1	1.00	GPI	17
2	1.00	EST, PGD	11
3	1.00	ADH, EST, GPI	4
4	0.95	HBDH, TPI	32
5	0.90	HBDH, PEP, TPI	23
6	0.80	HBDH ¹	5
7	0.70	HBDH, PGD	23
8	0.66	IDH ¹	6
9	0.64	HBDH ¹	11
10	0.50	PEP ¹	4
11	0.50	HBDH ¹	4
12	0.03	IDH ¹	32
13	0.00	ADH, HBDH, IDH	16
Mean	0.67		

¹ One locus provided unambiguous paternity information.

id beetle, *Tribolium castaneum* (LEWIS & AUSTAD 1990). The precedence values for at least eight of the families should be quite reliable since they are based on large numbers of offspring.

It is surprising that there were no sperm precedence values between 0.03 and 0.50 (Table 1). The reason for this discontinuous distribution is unknown. It is possible that it is due to females' preferential use of the sperm from one of the males, independent of mating order. It is unlikely that the two cases of first-male precedence were caused by a lack of spermatophore transfer by the second male for two reasons. First, in one of the families one offspring was definitely sired by the second male. Second, guarding behaviour, a very reliable indicator of spermatophore transfer (CONNER 1989), followed all matings. Both of these males had been kept in isolation from females for 2 to 3 weeks, so it is unlikely that their sperm were depleted by previous matings. A discontinuous distribution of sperm precedence values has also been reported in the soybean looper (*Pseudoplusia includens*), a noctuid moth (MASON & PASHLEY 1991).

The high variability in sperm precedence reported here means that sperm competition might affect male fitness in *B. cornutus*. If the variability is related to specific male traits, then this would create selection on the traits. In a preliminary analysis using the eight families that had more than 10 offspring, there were no significant correlations between the level of precedence and several measures of the body sizes of both males or the time interval between the matings (CONNER unpub.). The time interval between matings varied between 2 and 20 days (11.5 ± 2.1 , mean \pm SEM), and cannot be controlled for because it is determined by female receptivity. Another factor that may affect sperm precedence is copulation duration (DICKINSON 1986); I did not record copulation duration in this study because copulations are fairly short (generally < 10 min; CONNER unpub.) and duration is less

likely to be important to sperm precedence in an insect like *B. cornutus* that transfers sperm packaged in a spermatophore. To be definitive, these preliminary analyses need to be repeated with more than eight families and more male traits analyzed. Future studies of sperm competition need to focus not only on intraspecific variability in sperm precedence, but possible reasons for this variability (see DICKINSON 1986, LEWIS & AUSTAD 1990, and SIMMONS & PARKER 1992 for examples of this approach).

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