J. New York Entomol. Soc. 99(4):637–642, 1991

A GENETIC MARKER FOR INVESTIGATING PATERNITY AND MATERNITY IN THE BURYING BEETLE *NICROPHORUS ORBICOLLIS* (COLEOPTERA: SILPHIDAE)

STEPHEN T. TRUMBO AND ANTHONY J. FIORE
Department of Biology, State University of New York,
Binghamton, New York 13902-6000

Abstract.—A purebred "spotless" line of *Nicrophorus orbicollis* was produced by inbreeding. Spotless beetles completely lack orange markings on the basal portion of the elytra. The spotless trait appears to be largely under the influence of a single gene and is an excellent genetic marker for paternity or maternity in a variety of competitive breeding situations. In the laboratory, individuals possessing the spotless trait were as reproductively successful as normally marked beetles. The spotless marker was used to demonstrate that males which pair with a female achieve a high level of paternity. Paternity remained high in a second brood even when the male was separated from the female in the interval between reproductive attempts.

The ability to assign paternity and maternity has become nearly essential in behavioral studies of reproductive success. Three prominent techniques include: sterilization of males, molecular genetic comparisons (e.g., electrophoresis and DNA fingerprinting) and phenotypic markers. In many situations, phenotypic markers are ideal because subjects do not have to be handled or sacrificed and the employment of the technique has limited effect on behavior or vigor.

One important application of genetic markers has been to the study of sperm competition, a process in which ejaculates of more than male compete for fertilization of an egg (Parker, 1970). In the majority of insects investigated the last male to mate fathers a high proportion of the brood (Parker, 1970; Gwynne, 1984). A strong correlation between paternity and male parental care has been noted (Ridley, 1978; Alexander and Borgia, 1979) despite the fact that there are no theoretical reasons why a high level of paternity itself should promote paternal care (Maynard Smith, 1978; Werren et al., 1980). Once paternal care has evolved, however, paternity-enhancing mechanisms which require extended male-female contact can be selected (Werren et al., 1980; Knowlten and Greenwell, 1984).

The need to employ genetic markers to investigate sperm competition and other aspects of reproductive competition in *Nicrophorus* is evident because of the complexity of social interactions. Males and females arrive at small vertebrate carcasses and compete intrasexually for the right to breed. The dominant male and female bury the carcass, remove any hair or feathers and roll the carcass into a ball (Pukowski, 1933). Courtship is minimal and mate choice appears to be entirely passive (Milne and Milne, 1976; Otronen, 1988). If a male fails to discover the carcass a female will breed on her own using stored sperm (Bartlett, 1988; Scott, 1989; Trumbo, 1990a). Females have acquired sperm by copulating with males that emit pheromones in the absence of a carcass or by copulating with males on large carcasses where only feeding occurs (Müller and Eggert, 1987; Eggert and Müller, 1990). When more than one

male discovers the carcass, the subordinate male can adopt a satellite strategy and obtain some reproductive success despite being forced off the carcass by the dominant male (Bartlett, 1988). Subordinate females that are displaced from the carcass also can achieve some reproductive success by brood parasitism (Müller et al., 1990). The resident male fathers 92% of offspring in *N. vespilloides* by copulating repeatedly with the female prior to and throughout oviposition (Müller and Eggert, 1989). Once larvae appear on the carcass, they are fed and guarded by both parents (Bartlett, 1988; Scott, 1990; Scott and Traniello, 1990). The male usually deserts before the female and both sexes can attempt reproduction a second or third time in the breeding season.

In this paper we describe a phenotypic marker ('spotless') for *N. orbicollis* Say that can be used to determine either paternity or maternity, examine the genetic basis of the marker, compare the reproductive success of two stocks possessing alternative genetic markers and apply the marker in an initial sperm competition experiment.

METHODS

Genetic basis of the marker. A single *Nicrophorus orbicollis* male completely lacking orange basal markings of the elytra was caught on a mouse carcass at The University of Michigan Biological Station in 1986 and subsequently bred to three normally marked females. All beetles were reared and bred at 19–22°C and a 15L:9D cycle. These hybrid offspring were crossed and the resulting F_2 population contained approximately one-quarter spotless individuals. Additional normal × normal crosses were made to produce an F_2 laboratory population of normally marked beetles. Nine different types of crosses were then made using these F_2 stocks and hybrid F_3 individuals (hybrid individuals always had some degree of marking on the basal portion of the elytra). Females were isolated a few days after adult emergence and paired with males 1 day prior to trials. These pairs were placed in 8 × 15 × 30 cm containers filled with soil and provided a mouse carcass (21–30 g). Progeny from a total of 87 crosses were reared to the adult stage and scored as spotted (having some degree of basal marking) or spotless.

Comparative reproductive success. The spotless stock was maintained through inbreeding and outcrossed in 1987 to field caught beetles. The resulting hybrid progeny were crossed to start a new spotless laboratory population. The outcrossing procedure was an attempt to avoid inbreeding depression in our laboratory populations. Additional normal × normal crosses were made using field caught beetles to start a new laboratory population of normally marked beetles. To compare the reproductive performance of these two new stocks, 17 normal × normal crosses and 18 spotless × spotless crosses were made using 25–30 g mouse carcasses as a breeding resource for each pair. Larvae were counted and the mass of the brood was determined at the time larvae dispersed from the nest.

Paternity of the resident male. The spotless stock was again outcrossed and a new spotless laboratory population was started from progeny of hybrid × hybrid crosses. The laboratory population of normally marked beetles was maintained and kept in reproductive synchrony with the spotless population. A few days after adult emergence groups of 5 spotless females were placed into containers with either 5 normal males or 5 spotless males. At 22–28 days females were paired with a male of the

Table 1. Frequency of phenotypes resulting from test crosses.

Presumed genotype of male parent[1]	Presumed genotype of female parent	Number of crosses	Phenotype of offspring				G[2]
			Spotless male	Spotless female	Normal male	Normal female	
spl-spl	*spl-spl*	18	106	122	0	0	—
nor-nor	*nor-nor*	6	0	0	30	33	—
spl-nor	*spl-nor*	20	28	29	81	92	0.01*
spl-spl	*nor-nor*	6	0	0	32	22	—
nor-nor	*spl-spl*	5	0	0	24	28	—
spl-spl	*spl-nor*	14	34	43	26	34	2.11**
spl-nor	*spl-spl*	10	30	28	28	37	0.40*
nor-nor	*spl-nor*	3	0	0	16	19	—
spl-nor	*nor-nor*	4	0	0	21	27	—

[1] *spl-spl* were spotless beetles; *nor-nor* were normally marked beetles; and, *spl-nor* were offspring of known *spl-spl* × *nor-nor* crosses.

[2] G values computed by comparing the observed frequency of phenotypes with the expected frequency based on a one gene model for the trait: * $P > 0.2$; ** $P > 0.1$.

alternative genetic marker and each pair was provided a mouse carcass (21–24 g) on which to breed. Larvae were counted and weighed as before and paternity was determined after adult emergence. Parents were separated after larvae dispersed and 5 days later each isolated female was provided a second 21–24 g carcass. Resulting progeny were counted and weighed at larval dispersal and paternity was determined by examining offspring at the adult stage.

RESULTS

All types of crosses involving normal, spotless and hybrid beetles produced offspring whose phenotype could be scored as either spotted or spotless. The distribution of phenotypes among offspring suggests that a single gene is primarily responsible for the spotless mutation such that a homozygote individual completely lacks orange markings on the basal portion of the elytra (Table 1). Heterozygote individuals, however, varied considerably in the degree of basal marking and could not be distinguished reliably from homozygote normal beetles.

In the second experiment, 16 of 17 normal × normal crosses and 16 of 18 spotless × spotless crosses produced offspring. Neither the mean (±SE) number of larvae at dispersal (12.88 ± 1.28 vs. 12.56 ± 1.29, F = 0.11, ns) nor the mean (±SE) mass of broods (5.49 ± 0.51 g vs. 5.28 ± 0.35 g, F = 0.29, ns) differed between normal × normal and spotless × spotless crosses, respectively.

A male that pairs with a female on the carcass and copulates throughout the oviposition period achieves a high degree of paternity (93% of total offspring in first broods, Table 2). The genotype of a paired male, and the genotype-reproductive attempt interaction were not related significantly to either the number of larvae or the mass of the brood. Second reproductive attempts had significantly more larvae and a larger brood mass than first reproductive attempts. Paternity of paired males remained high (96% of total offspring in second broods) even though females were

Table 2. Success in first and second reproductive attempts for *spl-spl* females initially paired with normally marked (*nor-nor*) or spotless (*spl-spl*) males.

	nor-nor male		*spl-spl* male	
	First attempt	Second attempt	First attempt	Second attempt
Mean (±SE) number of offspring	9.67 (1.36)	13.63 (0.71)	10.00 (1.05)	12.67 (0.62)
Mean (±SE) mass of brood	4.20 (0.67)	5.79 (0.27)	4.74 (0.40)	5.29 (0.20)
Proportion of mixed broods	0.33	0.25	0.22	0.33
Mean proportion of brood attributed to the paired male	0.92	0.98	0.91	0.95

Two-way ANOVAs performed to test effects of Male Genotype (MG) and Reproductive Attempt (RA). Number of offspring: $F_{MG} = 0.10$, ns; $F_{RA} = 11.00$, $P = 0.002$; $F_{MG \times RA} = 0.42$, ns. Mass of brood: $F_{MG} = 0.00$, ns; $F_{RA} = 6.03$, $P = 0.02$; $F_{MG \times RA} = 1.43$, ns. Proportion (arcsin transformed) of brood attributed to paired male: $F_{MG} = 0.05$, ns; $F_{RA} = 0.30$, ns; $F_{MG \times RA} = 0.15$, ns.

isolated from males between reproductive attempts and subsequently reproduced on their own.

DISCUSSION

The spotless trait is an unambiguous genetic marker that permits the identification of paternity or maternity in competitive breeding situations involving appropriate individuals. The trait is largely controlled by a single gene although variation among heterozygote and homozygote normal beetles suggests that additional genes or the environment are involved in determining the extent of marking on the basal portion of the elytra. The use of this genetic marker has been employed to demonstrate that both male and female burying beetles obtain reproductive benefits following infanticide (Trumbo, 1990b). Similar elytral pattern markers have been used to demonstrate intraspecific brood parasitism in *N. vespilloides* (Müller et al., 1990). Anderson and Peck (1986) examined variation in elytral patterns across North American species of *Nicrophorus* and found that melanic forms were most common on the Pacific Northwest coast. They speculate that melanism might play a role in thermoregulation in localities with lower levels of solar radiation. Presumably the tradeoff of melanism is less protection from predators since the bright orange marks on the elytra are thought to function as aposematic warning coloration. If true, elytral pattern variation is an excellent marker for comparing reproductive success in laboratory situations not involving predation.

Males of *N. vespilloides* and *N. orbicollis* that pair with a female obtain a high level of paternity (Bartlett, 1988; Müller and Eggert, 1989) as is common among insects with paternal care (Alexander and Borgia, 1979). As in male brooding water bugs, the paternity-enhancing mechanism is repeated copulation before and throughout oviposition (Smith, 1979; Müller and Eggert, 1989). This strategy is especially effective when females mate with other males between copulatory attempts by the dominant male (Thornhill and Alcock, 1983), a situation that likely occurs in *Nicrophorus* (Wilson and Fudge, 1984; Bartlett, 1988).

When competitors are not present, a *Nicrophorus* female that attempts to breed

on her own achieves equal or greater reproductive success than pairs (Bartlett, 1988; Scott, 1989; Trumbo, 1990a). To reproduce successfully without the help of a male, a female must periodically obtain fresh sperm from a male because sperm become inviable; in *N. vespilloides,* 16% of sperm were inviable after 14 days and 43% were inviable after 21 days (Eggert and Müller, 1989). It is unclear whether the high level of paternity that males obtained in the second reproductive attempt was due to a lack of sperm mixing or the inviability of sperm from previously mated males (sperm from previous males were approximately 16 days old at the start of the second reproductive attempt). The more common pattern in insects is for mixing to occur with time (Schlager, 1960; Siva-Jothy and Tsubaki, 1989). A high degree of paternity is obtained in some species by flushing or removal of previously deposited sperm (Waage, 1979; Parker, 1984) but this does not appear compatible with the male burying beetle strategy of mating as many as 100 times during the oviposition period (Müller and Eggert, 1989). By whatever mechanism, a male that stays with a female throughout oviposition might obtain an additional reproductive benefit if the female completes the present reproductive attempt and subsequently breeds on her own.

Second reproductive attempts produced more larvae and a larger brood than first reproductive attempts, contrary to results reported previously (Scott and Traniello, 1990; Trumbo, 1990c). Scott (1989) found that the presence of a male decreased reproductive success if the male was confined to the nest area until larvae dispersed, but a male does not have a negative effect if he is removed or allowed to escape by day 9 (Scott, pers. comm.; Trumbo, 1991). Since the male was retained in breeding containers in our study, this might provide one explanation for lower reproductive success in first reproductive attempts.

ACKNOWLEDGMENTS

We thank Anne Clark and Sue Trumbo for reviewing earlier versions of this paper. This work was supported by The Mason Farm Biological Reserve at The University of North Carolina at Chapel Hill, The University of Michigan Biological Station and by NSF BSR grant 89-06183.

LITERATURE CITED

Alexander, R. D. and G. Borgia. 1979. On the origin and basis of the male-female phenomenon. Pages 417–440 *in:* M. S. Blum and N. A. Blum (eds.), Sexual Selection and Reproductive Competition in Insects. Academic Press, New York.

Anderson, R. S. and S. B. Peck. 1986. Geographic patterns of color variation in North American *Nicrophorus* beetles (Coleoptera: Silphidae). J. Nat. Hist. 20:282–297.

Bartlett, J. 1988. Male mating success and paternal care in *Nicrophorus vespilloides.* Behav. Ecol. Sociobiol. 22:429–434.

Eggert, A-K. and J. K. Müller. 1989. Uni- and biparentale Brutpflege bei *Necrophorus vespilloides.* Verh. Dtsch. Zool. Ges. 82:318.

Eggert, A-K. and J. K. Müller. 1990. Mating success of pheromone-emitting *Necrophorus* males: do attracted females discriminate against resource owners? Behaviour (in press).

Gwynne, D. T. 1984. Male mating effect, confidence of paternity, and insect sperm competition. Pages 117–150 *in:* R. L. Smith (ed.), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, Orlando, Florida.

Knowlten, N. and S. R. Greenwell. 1984. Male sperm competition avoidance mechanisms: the influence of female interests. Pages 62–85 *in:* R. L. Smith (ed.), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, Orlando, Florida.

on her own achieves equal or greater reproductive success than pairs (Bartlett, 1988; Scott, 1989; Trumbo, 1990a). To reproduce successfully without the help of a male, a female must periodically obtain fresh sperm from a male because sperm become inviable; in *N. vespilloides,* 16% of sperm were inviable after 14 days and 43% were inviable after 21 days (Eggert and Müller, 1989). It is unclear whether the high level of paternity that males obtained in the second reproductive attempt was due to a lack of sperm mixing or the inviability of sperm from previously mated males (sperm from previous males were approximately 16 days old at the start of the second reproductive attempt). The more common pattern in insects is for mixing to occur with time (Schlager, 1960; Siva-Jothy and Tsubaki, 1989). A high degree of paternity is obtained in some species by flushing or removal of previously deposited sperm (Waage, 1979; Parker, 1984) but this does not appear compatible with the male burying beetle strategy of mating as many as 100 times during the oviposition period (Müller and Eggert, 1989). By whatever mechanism, a male that stays with a female throughout oviposition might obtain an additional reproductive benefit if the female completes the present reproductive attempt and subsequently breeds on her own.

Second reproductive attempts produced more larvae and a larger brood than first reproductive attempts, contrary to results reported previously (Scott and Traniello, 1990; Trumbo, 1990c). Scott (1989) found that the presence of a male decreased reproductive success if the male was confined to the nest area until larvae dispersed, but a male does not have a negative effect if he is removed or allowed to escape by day 9 (Scott, pers. comm.; Trumbo, 1991). Since the male was retained in breeding containers in our study, this might provide one explanation for lower reproductive success in first reproductive attempts.

ACKNOWLEDGMENTS

We thank Anne Clark and Sue Trumbo for reviewing earlier versions of this paper. This work was supported by The Mason Farm Biological Reserve at The University of North Carolina at Chapel Hill, The University of Michigan Biological Station and by NSF BSR grant 89-06183.

LITERATURE CITED

Alexander, R. D. and G. Borgia. 1979. On the origin and basis of the male-female phenomenon. Pages 417–440 *in:* M. S. Blum and N. A. Blum (eds.), Sexual Selection and Reproductive Competition in Insects. Academic Press, New York.

Anderson, R. S. and S. B. Peck. 1986. Geographic patterns of color variation in North American *Nicrophorus* beetles (Coleoptera: Silphidae). J. Nat. Hist. 20:282–297.

Bartlett, J. 1988. Male mating success and paternal care in *Nicrophorus vespilloides.* Behav. Ecol. Sociobiol. 22:429–434.

Eggert, A-K. and J. K. Müller. 1989. Uni- and biparentale Brutpflege bei *Necrophorus vespilloides.* Verh. Dtsch. Zool. Ges. 82:318.

Eggert, A-K. and J. K. Müller. 1990. Mating success of pheromone-emitting *Necrophorus* males: do attracted females discriminate against resource owners? Behaviour (in press).

Gwynne, D. T. 1984. Male mating effect, confidence of paternity, and insect sperm competition. Pages 117–150 *in:* R. L. Smith (ed.), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, Orlando, Florida.

Knowlten, N. and S. R. Greenwell. 1984. Male sperm competition avoidance mechanisms: the influence of female interests. Pages 62–85 *in:* R. L. Smith (ed.), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, Orlando, Florida.

Maynard Smith, J. 1978. The Evolution of Sex. Cambridge University Press, Cambridge.
Milne, L. J. and M. Milne. 1976. The social behavior of burying beetles. Sci. Am. 235: 84–89.
Müller, J. K. and A-K. Eggert. 1987. Effects of carrion-independent pheromone emission by male burying beetles (Silphidae: *Necrophorus*). Ethology 76:297–304.
Müller, J. K. and A-K. Eggert. 1989. Paternity assurance by "helpful" males: adaptations to sperm competition in burying beetles. Behav. Ecol. Sociobiol. 24:245–249.
Müller, J. K., A-K. Eggert and J. Dressel. 1990. Intraspecific brood parasitism in the burying beetle, *Necrophorus vespilloides* (Coleoptera: Silphidae). Anim. Behav. 40:491–499.
Otronen, M. 1988. The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*). Ann. Zool. Fennici 25:191–201.
Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45:263–281.
Parker, G. A. 1984. Sperm competition and the evolution of animal mating strategies. Pages 1–60 *in:* R. L. Smith (ed.), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, Orlando, Florida.
Pukowski, E. 1933. Ökologische Untersuchungen an *Necrophorus* F. Z. Morphol. Ökol. Tiere 27:518–586.
Ridley, M. 1978. Paternal care. Anim. Behav. 26:904–932.
Schlager, G. 1960. Sperm precedence in the fertilization of eggs in *Tribolium castaneum*. Ann. Entomol. Soc. Am. 53:557–560.
Scott, M. P. 1989. Male parental care and reproductive success in the burying beetle *Nicrophorus orbicollis*. J. Ins. Behav. 2:133–137.
Scott, M. P. 1990. Brood guarding and the evolution of male parental care in burying beetles. Behav. Ecol. Sociobiol. 26:31–39.
Scott, M. P. and J. F. A. Traniello. 1990. Behavioural and ecological correlates of male and female parental care and reproductive success in burying beetles (*Nicrophorus* spp.). Anim. Behav. 39:274–283.
Siva-Jothy, M. T. and Y. Tsubaki. 1989. Variation in copulation duration in *Mnais pruinosa pruinosa* Selys (Odonata: Calopterygidae) 1. Alternative mate-securing tactics and sperm precedence. Behav. Ecol. Sociobiol. 24:39–45.
Smith, R. L. 1979. Repeated copulation and sperm precedence: paternity assurance for a male brooding water bug. Science 205:1029–1031.
Thornhill, R. and J. Alcock. 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge, Mass.
Trumbo, S. T. 1990a. Interference competition among burying beetles (Silphidae: *Nicrophorus*). Ecol. Entomol. 15:347–355.
Trumbo, S. T. 1990b. Reproductive benefits of infanticide in a biparental burying beetle, *Nicrophorus orbicollis*. Behav. Ecol. Sociobiol. 27:269–273.
Trumbo, S. T. 1990c. Brood size regulation in a burying beetle, *Nicrophorus tomentosus*. J. Ins. Behav. 3:491–500.
Trumbo, S. T. 1991. Reproductive benefits and the duration of paternal care in a biparental burying beetle, *Nicrophorus orbicollis*. Behaviour 117:82–105.
Waage, J. K. 1979. Dual function of the damselfly penis: sperm removal and transfer. Science 203:916–918.
Werren, J. H., M. R. Gross and R. Shine. 1980. Paternity and the evolution of male parental care. J. Theor. Biol. 82:619–631.
Wilson, D. S. and J. Fudge. 1984. Burying beetles: intraspecific interactions and reproductive success in the field. Ecol. Entomol. 9:195–204.

Received 30 October 1991; accepted 17 April 1991.