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Paternity assurance by "helpful" males: adaptations to sperm competition in burying beetles

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Summary. Male burying beetles invest parentally by participating in the burial of a carcass and in provisioning and guarding the larvae that come to the carcass. Since most of the females arriving at a carcass have stored fertile sperm within their spermathecae, sperm transferred by such "helpful" males must compete with other males' ejaculates for the fertilization of the female's eggs. We showed that these males are able to achieve a high level of paternity (mean = 92%). The mechanism they employ is a repeat-mating tactic, i.e., the female is mated very frequently shortly before and during oviposition. Repeated matings are essential for a high reliability of paternity, since single copulations result in the fertilization of only a very small proportion of the female's eggs.

Introduction

Since Parker (1970), many publications have dealt with sperm competition and paternity assurance in animals. Trivers (1972) was the first to indicate that in species with substantial paternal investment, males should have evolved adaptations to decrease their vulnerability to cuckoldry. In the following years, several authors restated that a correlation between male parental investment and certainty of paternity should exist (e.g., Thornhill 1979). Yet this initially accepted correlation was doubted repeatedly during the following years. In 1978 Maynard Smith stated that a general correlation between paternity and male parental care could not be derived from theoretical considerations. Other authors specified the conditions under which such a correlation could be expected: (1) the "sacrificing" of promiscuous copulations by parental males (Werren et al. 1980; Grafen 1980) or (2) an extreme time delay between broods due to a prolonged search by females for males (Parker 1984). The only model we know that does predict a correlation between male parental care and paternity – which is achieved by the influence of paternal investment on paternity and not vice versa – is the one developed by Knowlton and Greenwell (1984). Though this correlation can be observed in many species (see Gwynne 1984), the problem still remains controversial among theoreticians.

Male parental care in insects is a relatively rare phenomenon; most cases of male parental investment or nonpromiscuous mating effort consist in the transfer of nutrients or other substances to the female (see Gwynne 1984). Exceptions to this rule are, for example, male brooding water bugs (Smith 1979), woodroaches of the genus *Cryptocercus* (Seelinger and Seelinger 1983; Nalepa 1984, 1988), and burying beetles of the genus *Necrophorus*.

Burying beetles utilize small vertebrate carcasses as a larval food source; the carcass is buried and prepared by either a pair or a single female. Aggressive contests occur if intra- or interspecific competitors are present on the carcass. The female lays the eggs near the carcass and afterwards waits for the larvae, which approach the carcass by themselves, in order to feed them regurgitated carrion. The male, if present at the beginning, also stays with the brood for several days. More detailed descriptions of the natural history of burying beetles are given by Pukowski (1933) and Müller and Eggert (1988).

Like many of the female insects investigated to date, female burying beetles can store transferred sperm within a specialized storage organ, the spermatheca. Thus sperm competition for the fertilization of the female's eggs can occur any time a male mates with a non-virgin female whose sper-

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matheca contains fertile sperm from other males' ejaculates. Male burying beetles can be said to invest a considerable amount of time and energy in the female's offspring, since they help their mate with the interspecific defense, burial, and preparation of the carcass and with the feeding and guarding of the larvae. Such behavior pays in terms of fitness only if the male carrion beetle has evolved some kind of mechanism to increase the probability that the larvae he fosters are his own offspring. The aim of this paper is to present data on the effectiveness of paternity assurance in burying beetles as well as on the mechanism employed by males to pre-empt sperm from previous matings and from present competitors.

Methods

For our investigations we used burying beetles of the species *Necrophorus vespilloides*.

Sperm storage by females in the field

To find out how many females had stored fertile sperm, we used females that had been caught in baited pitfall traps in the absence of conspecific males. They were offered a carcass suitable for reproduction; 2 days and 4 days later we checked whether eggs had been laid or not. We defined a "reproductively active" female as a female that had started egg-laying within 4 days and a "non-reproductive" female as one that had not. Laboratory-bred females ($n > 100$), if they are sexually mature, always bury a carcass and lay eggs within 4 days. Eggs were collected and stored separately to check whether they had been fertilized or not. If a female had not started egg deposition within 4 days after she had been supplied a carcass, the female was dissected and the content of the spermatheca was checked under a microscope. The content was classified according to four categories (I, II, III, IV) varying from "none" to "many" motile sperm. A female was said to have stored fertile sperm only if (a) at least one larva had hatched from her eggs or (b) the sperm content of the spermatheca belonged to category III or IV.

Paternity tests

As a genetic marker for paternity tests we used the coloration of the beetles' elytra, which is determined by several genes. By inbreeding we had managed to establish two laboratory strains with clearly distinguishable colors of elytra: "Light" (L) and "Dark" (D). Beetles of the L phenotype almost completely lack the black band that is present in wild-type individuals; D individuals show an extreme extension of this black patch so that the posterior half of the elytra is almost completely black. The offspring of individual females (either D or L) that have been inseminated by both D and L males can clearly be distinguished according to their father's phenotype (Müller and Eggert 1988). Fertilization rates of the individual males used were examined before and after each experiment. Since male fertilization rates with wild-type females and females from the inbred strains were not significantly different, both kinds of females were used for fertility tests. Only those experiments were considered in which both males used had fertilization rates of more than 80% both before and after the experiment.

Single copulations. To test the effect of a definite number of copulations on paternity in a situation where sperm competition can occur, we used females whose spermathecae were filled with viable sperm of a male of one color type. This was easily achieved by keeping the female together with a male of this color type for 24 h. (Females [$n = 10$] dissected afterwards had spermathecae filled with motile sperm.) After these 24 h, a male of the other color type was allowed to mate with this female one, four, or seven times. A copulation was defined as intromission of the aedeagus; all observed copulations lasted longer than 20 s. Since immediately after a mating males do not make further attempts to mount the female, 30 min was used as the minimum time between two successive copulations. After these matings, the female was immediately supplied with a carcass suitable for reproduction. The larvae hatching from the female's eggs were raised by "nurses", i.e., females who had laid eggs and were waiting for their own larvae to come to the carcass. This was done to exclude effects caused by female preferences for a certain kind of larvae. Larvae from one clutch were raised by more than one nurse in order to reduce sib-competition for food among the larvae.

"Ad libitum" copulations by paternal males. As in the above experiment, we used mated females that had been kept together with a male for 24 h and, therefore, had spermathecae containing D or L sperm. In this experiment, however, the female was supplied with a carcass after these 24 h, and the second male was allowed to copulate as often as he wanted or could during the burial of this carcass. This second male was present until the first larva had hatched from the female's eggs. Again, the larvae were raised by nurses.

Mechanism of paternity assurance

Video tape recordings were used to investigate the behavior of males during burial and preparation of a carcass. A pair of laboratory-bred (F1 from field catches), sexually mature beetles was put into a glass container (∅10 cm) covered on the bottom with moist peat. Twelve h later, a dead mouse (7–8 g) was added. From that time on, the beetles' behavior was continuously recorded for 72 h. After an initial 12-h light phase, red light was used for recording. In complete darkness as well as under red light, the beetles show their normal reproductive behavior even though they cannot bury the carcass completely. In contrast, daylight makes them show obvious disturbance reactions and leads to continuous attempts to conceal the carcass completely.

It might be argued that the frequency of copulations observed under the above experimental conditions was unnatural, since the small container induced frequent encounters of the pair as soon as the beetles became active. In order to recognize whether the number of copulations was merely an effect of keeping the beetles so closely together, we carried out a control experiment that consisted of continuous 48-h recordings of the same pairs of beetles in the same type of container, but in the absence of a corpse. These experiments were carried out under the same light rhythm (16/8, L/D) the tested individuals had experienced before.

Results

Sperm storage by females in the field

Out of 191 tested females, 143 (75%) had stored fertile sperm according to our definition. Thus it

Table 1. Proportion of the offspring fathered by the second male under different experimental conditions (X = number of tested females; n = number of tested offspring)

Possibilities to copulate for		X	n	offspring of male 2	
male 1	male 2			%	range
24 h	1 copulation	13	302	11%	0– 21
24 h	4 copulations	11	214	19%	0– 44
24 h	7 copulations	4	88	50%	38– 76
24 h	during burial	15	360	92%	76–100

might seem that males had a considerable chance of meeting females without viable sperm at a carcass. However, if we consider only reproductively active females, 98 out of 105 such females (93%) proved to have stored viable sperm. For a discussion of paternity we can exclude non-reproductive females from our considerations. Thus we can say that within the relevant part of the female population, the majority of females are mated and have stored fertile sperm. Males that help a female bury a corpse obviously are in a situation where sperm competition is to be expected.

Of the non-reproductive females, only 52% had stored motile sperm (45 out of 86). The difference between reproductive and non-reproductive females was statistically significant ($P < 0.001$, χ^2-test). Most of the non-reproductive females are presumably either in reproductive diapause (in early spring), or they are sexually immature second-generation individuals (in July and August).

Degree of paternity after single copulations

Unlike many other insect species, in *N. vespilloides* the last male to mate does not have an advantage over previously mating competitors. Single matings yield only a very poor result for the last male; in our experiment a mere 11% of the female's eggs were his offspring (Table 1). Four or seven copulations by the last male did not guarantee more than 20% and 50% paternity success, respectively. These results show that a male burying beetle cannot achieve substantial paternity assurance by mating with a female once or only a few times. Yet there is a significant correlation between the number of copulations and the proportion of offspring fathered by a certain male (Spearman rank correlation: $rs = 0.64$, $P < 0.001$).

Degree of paternity after joint burial of a carcass

When males are allowed to mate with females at a carcass as often as they want or can, the results are quite different from the outcome of single copulations. In 15 cases tested, the males were able to fertilize a mean proportion of 92% of the female's eggs (Table 1). Although males are not completely successful in ensuring their paternity, they do reach a high level of sperm precedence in a situation in which they support the female in her reproductive effort. Paternity in this case is significantly higher ($P < 0.01$, U-test) than after one, four, or even seven copulations.

Mechanism of paternity assurance

The mechanism by which the males manage to achieve this high level of sperm precedence becomes clear from the results of the video recordings. Males that encounter a female in the absence of a carcass copulate about 29 times during 24 h of observation (SD = 8, $n = 8$ pairs). When each day is divided into six intervals of 4 h each, it becomes obvious that copulation frequency does have a certain daily rhythmicity (Fig. 1a).

If the same pairs are supplied with a carcass, copulations become significantly more frequent during the first 24 h ($\bar{x} = 70$, SD = 7, $n = 8$ pairs; Wilcoxon matched pairs signed rank test: $P < 0.01$). It is obvious that most of the copulations on a carcass take place during this initial period (Fig. 1b). Females also lay the bulk of their clutch during this time ($n = 8$, unpublished data). Mating frequency decreases during the second 24 h and reaches a minimum value of almost zero at the time the larvae hatch and approach the carcass (after 68 to 72 h). However, we do not know whether the male obtains any information from the female about the exact time of egg laying.

The rhythmicity that was observable without carrion was completely lost in the presence of carcasses. This may have been partly due to the light conditions used which were identical only during the first 20 h of observation. The duration of copulations measured under field conditions was the same in the presence or absence of a carcass. It varied from 20 to 90 s, with a mean of 52 s (SD = 18 s, $n = 64$).

Discussion

Nearly all of the reproductively active females of *N. vespilloides* had stored fertile sperm within their spermathecae. Scott and Traniello (1987), who investigated *N. tomentosus*, suggest that for *Necrophorus* females "in the absence of a male, the stimulus to bury may be sufficient motile sperm". Thus it may be argued that our results on the spermathe-

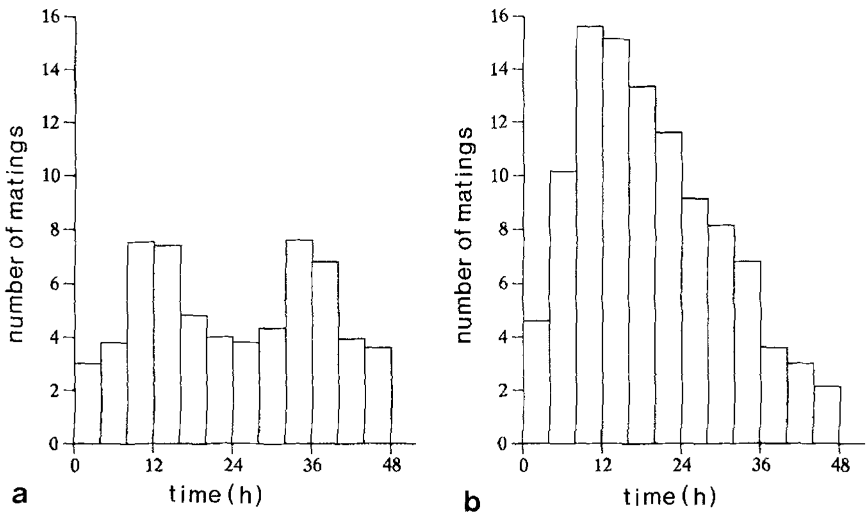

Fig. 1 a, b. Frequency of matings during 4-h intervals in pairs without a corpse (**a**), and in pairs on a corpse (**b**). Values figured are mean values calculated from $n=8$ pairs each

cal contents and reproductive status of females can be interpreted in a different way. If females without sufficient fertile sperm did not start egg deposition on carcasses at all or only with a substantial time delay, then we would expect a result similar to the observed one. However, females of *N. vespilloides* do not seem to require a storage of sufficient fertile sperm for burial of a carcass or egg deposition, since laboratory-raised sexually mature, but virgin females hardly ever fail to bury a suitable carcass and lay eggs within 4 days (personal observation). Moreover, Scott and Traniello (1987) admit that even in *N. tomentosus* "not all females which buried and had developed ovaries had many motile sperm". This means that a storage of sufficient fertile sperm is not essential for female egg deposition.

Storage of sufficient numbers of fertile sperm by sexually mature females could reasonably be expected in a species whose females are able to reproduce without the assistance of a mate (Pukowski 1933; Wilson and Fudge 1984). Females that store fertile sperm do not forego the opportunity of successful reproduction when they remain alone at a carcass. However, at the present time there are no reliable data available as to the frequency of females raising their brood alone. The females can gain fresh sperm from males they meet on carcasses or from pheromone-emitting ("sterzeling") males (Müller and Eggert 1987). This means that sperm transferred by a male on a carcass must compete with sperm from previous matings stored within the female's spermatheca. Another cause of sperm competition in this situation is the presence of conspecific competitors. Males other than the eventual winner may be present on a carcass or in its close proximity and will copulate with the female as often as possible (Dressel 1987).

Paternity tests show that male burying beetles obviously have evolved adaptations to this competitive situation, as expected (Parker 1970). But males cannot achieve a high certainty of paternity by mating once or only a few times, since the outcome of single copulations is relatively poor for the last mating male. In many insect species, the last male can fertilize 50% or more of the female's eggs by a single copulation (Gwynne 1984), whereas in *N. vespilloides*, the 50% level is reached only after seven consecutive matings of the last male. However, in a reproductive situation on a carcass, the male does achieve a high level of paternity by copulating very frequently before and during the period of egg deposition. This example illustrates the importance experimental design has for the results of paternity experiments. If one knows only the results of single-copulation paternity tests, one might ask why the male burying beetle is unable to ensure paternity.

Many insect species have mating patterns completely different from *N. vespilloides*; e.g., a single copulation is often sufficient to fertilize the female's lifetime production of eggs (Parker 1970). One may ask why the *N. vespilloides* male does not reduce the number of copulations and extend the duration of every single mating instead. Several factors may have been important for the origin and maintenance of this pattern: (1) female interests to make the partner stay near the carcass, (2) an insufficient capacity of the male to produce large amounts of sperm, (3) the necessity of rapid concealment of the carcass, (4) the risk of subsequent copulations by other males on or near the carcass (Dressel and Müller 1988), or (5) necessity of a flexible copulation pattern that can be adapted to different situations, since copulations take place also in the absence of a carcass suitable for reproduction (personal observations).

The data presented here cannot support any of the models mentioned in the introduction. We do not know how male participation in parental care evolved in burying beetles. However, the results show that the widely accepted assumption that paternity for parental and nonparental males should be about equal is most likely not valid for burying beetles. In *N. vespilloides*, parental males are able to increase their certainty of paternity on a carcass due to the special circumstances of the situation in which parental investment occurs, i.e., permanent access to the female shortly before oviposition. In a non-parental situation, i.e., when the sexes meet on a carcass serving as an adult food source or when a female has approached a pheromone-emitting male, males do not have the opportunity to mate with the female as often as on a carcass used for reproduction, and egg deposition, if it takes place at all, will occur at some unpredictable future time. Thus, paternity of nonparentals will probably remain low, though parentals manage to increase their certainty of paternity. Under these conditions it is easy to imagine that paternity assurance mechanisms by parental males would rapidly be established within the population. We feel that theoretical biologists concerned with the problem of evolution of paternal care should take into consideration that differential paternity for parental and nonparental males might be a phenomenon that is more widespread than they assumed.

When looking at the results of our study with respect to the success of the male's behavior, we must keep in mind that our experiments cover only part of the natural situation. We did not introduce any competing males while the pair was on the carcass, whereas under field conditions the male often has to cope with the presence of like-sexed conspecifics on a carcass. Further investigation needs to be done on male paternity in a competitive situation on a carcass.

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