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MATING SUCCESS OF PHEROMONE-EMITTING NECROPHORUS MALES: DO ATTRACTED FEMALES DISCRIMINATE AGAINST RESOURCE OWNERS?

by

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Introduction

Pheromone emission in male burying beetles was first described by PUKOWSKI (1933) who had observed a conspicuous behaviour of males that had remained alone on a carcass: They climb an elevated place and adopt a 'typical, very surprising posture' (translation by the authors) with the head held down and the extremely extended abdomen pointing up. This posture is maintained for several hours, and only the tip of the abdomen is moved slightly up and down or in circles. PUKOWSKI already suspected that the male burying beetle in this situation produces a 'sex and species specific scent' that 'attracts females'. In analogy to a well-known term for scent attraction in honey bees she termed the behaviour 'sterzeln'. Some years later, she published a description of a direct observation of a female *N. vespillo* approaching and meeting a 'sterzeling' male (MOSEBACH, 1936). But pheromone emission in burying beetles is not restricted to the situation in which a male has found a carcass. In a pitfall study by MÜLLER & EGGERT (1987) males of three Middle European *Necrophorus* species were used as baits and proved to be attractive for conspecifics and congenerics even in the absence of carrion. Recent publications by BARTLETT (1987) and EGGERT & MÜLLER (1989) have provided further evidence that attraction of females is due to chemical communication.

The aim of the present paper is to show the effectiveness of male pheromone emission in *Necrophorus vespilloides* and to compare the behaviour of females towards the two types of males: (1) males that emit

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¹) We thank Mr. Alan CONNOR for correcting the English.

pheromones on or near a carcass, and (2) males that emit pheromones without having found a carcass.

Methods

a. *General outline.*

Pheromone-emitting males of the species *N. vespilloides* and the females attracted by these males were observed in the field. Some of the males had not buried a carcass, others had, but had been removed from the carcass after they had started pheromone emission. By having both types of males emit pheromone in the absence of carrion, we wanted to find out whether attracted females are able to distinguish males that have buried a carcass from males that have not. We did not perform a control experiment in which the carcass was present during pheromone emission, because the behaviour of pairs on a carcass is known (*e.g.*, MÜLLER & EGGERT, 1989). With some of the males that had buried a carcass, the male and the attracted female were given this carcass after the female had stayed with the male for some time. This was done to test whether factors other than the absence of the buried carcass had caused the females to reject copulations.

b. *Experimental design.*

The study was carried out during summer 1987 (May to early September) in a deciduous forest in northern Germany. Observations started during late afternoon (5 p.m. to 7 p.m.) and lasted until complete darkness. Oral descriptions of the observed behaviour were recorded with a mini cassette recorder and analysed the following day. The pheromone-emitting males used were laboratory-bred, sexually mature individuals.

Observations were performed on arenas (ø 62 cm) with a 5-cm high rim which prevented uncontrolled escaping of beetles. The floor of the arena was covered with moist peat. Outside observation periods the males were enclosed by covering the central part of the arena with a plastic strainer (ø 16 cm). The males could bury themselves in a peat-filled tin whose upper rim was on a level with the floor of the arena.

Males were submitted to either of two different treatments: (1) The male was simply left enclosed in the arena overnight, and by the next afternoon he had usually started pheromone emission on top of a small piece of wood standing out in the centre of the peat-filled tin. The strainer could then be removed and observation begin. (2) In the evening the male was offered a mouse carcass on top of the peat-filled tin. Usually he had buried the mouse by the following afternoon and then started pheromone emission on top of a piece of wood beside the tin. The male usually continued pheromone emission while the piece of wood was transferred to another arena and placed into the centre of this 'new' arena. Observation began after the transfer of the male. In some of the cases in which a female had been attracted by such male and stayed for 40 minutes or more the carcass was laid into the arena to observe the beetles' behaviour after detection of the carcass.

Copulation attempts were regarded as 'successful' if the male kept his aedeagus inside the female for at least 10 seconds. Correspondingly, 'unsuccessful' attempt means that the male mounted the female and tried to insert his aedeagus, but either failed completely or managed to keep the aedeagus inside the female for less than 10 seconds. This value is to some extent an arbitrary one, but males that succeed in inserting their aedeagus for less than 10 seconds usually repeat their copulation attempt, whereas successful copulation attempts are followed by a period of at least 10 minutes without any attempts to mate again with the same female. Direct observation of sperm transfer is not possible.

The number of cases (n) considered with each of the measured variables is not always the same, because field conditions did not always allow exact determination of every single variable, and because the behaviour was taken into consideration only as long as both partners had not been subject to any kind of interference.

Natural history of burying beetles.

Burying beetles reproduce on small vertebrate carcasses. Either a pair or a female alone buries the carcass and prepares it. The female lays eggs into the soil near the carcass and afterwards stays on the carcass, waiting for the larvae, which are then fed regurgitated carrion. If a male is present in the initial burying phase, he stays with the female and the brood for several days. The female usually stays with the brood until the carcass has been consumed and the larvae leave the brood chamber to pupate. Females that stay alone on a carcass start reproduction on their own, utilizing stored sperm. Males in this situation start pheromone emission near the carcass, sometimes after having started burial of the carcass.

Pheromone emission is restricted to a species-specific time of the day (MÜLLER & EGGERT, 1987), no matter whether the male has found a carcass or not. Pheromone attraction is neither entirely sex nor species specific; conspecific males and individuals of other *Necrophorus* species are also attracted.

Results

1. Effectiveness of pheromone attraction: Are males without carrion more attractive?

Pheromone-emitting males were observed on a total of 91 days. On 71 of these days (78%) they attracted and met at least one conspecific female. On 7 days (8%) they attracted conspecifics that left without having met the male. We assume that the failure of successful attraction, at least on entirely unsuccessful days (= without any attraction of conspecifics: 13 days = 14%), is due to unfavourable weather conditions. All of these days were either relatively cold (temperature 14-15°C or below) or extremely calm with no observable motions of the air. Entirely unsuccessful days are not considered in the following comparisons.

Males that had not buried a carcass (subsequently called 'have-not' males) attracted and met significantly more females per evening than did

TABLE 1. Attractiveness and duration of pheromone emission in 'have' and 'have-not' males (completely unsuccessful days not considered)

	Male 'haves' (35 days)	Male 'have-nots' (43 days)
Number of females that met the male per evening (mean ± s.d.)	0.97 ± 0.57	1.84 ± 1.27
	$p < 0.01$	
Duration of scent emission per evening (mean ± s.d.)	1:07 ± 0:35 hrs	2:09 ± 0:56 hrs
	$p < 0.001$	
Number of females that met the male per hour of scent emission	0.86	0.85

Statistics: U-test.

males that had buried a carcass (subsequently called 'have' males) (Table 1). From this result it might seem that 'have not' males are more effective in their attraction of females or that females prefer 'have-not' males over 'haves'. But a closer look shows that the duration of daily pheromone emission was longer in 'have-not' males (Table 1). 'Have-not' males attracted more females per evening than 'haves', but both types of males were equally successful with respect to the number of females attracted per unit time of pheromone emission.

If the attracted female did not land in the very close proximity of the male, the two beetles ran through the arena, hesitating from time to time, maybe climbing an elevated point, and always waving their antennae. If no contact had occurred after some minutes, the female left without having met the male.

2. Behaviour after male-female contact: Do females discriminate against resource owners?

As soon as male and female met, the male tried to mount the female and copulate with her. The time delay between first contact and the male's first attempt to penetrate the female was on average 14 seconds (n = 105, s.d. = 25 s). There was no significant difference between the treatment groups with and without carcasses (U-test, p = 0.364).

The first noticeable difference in the behaviour of females towards 'have' and 'have-not' males occurred after the first contact, when the male tried to inseminate the female. If a female accepts copulation, she does not try to escape from the male and bends the tip of her abdomen slightly upwards. Reluctant females try to escape from the male's grip or bend their abdomen downwards, thus preventing intromission of the aedeagus. But even reluctant females cannot always prevent copulation.

Most of the females attracted to 'have-not' males readily accepted copulation (Table 2), and with even more of them, the male's first copulation attempt was successful. In contrast to this, only one of the observed females accepted copulation by a 'have' male, but 22% of the males' first copulation attempts were successful. If a male is not allowed to copulate upon the first attempt, he usually tries to follow the female and mount her again to start another attempt. Unsuccessful copulation attempts lasted on average 17 seconds (n = 131, s.d. = 14 s), measured from the male's first attempt to insert his aedeagus to the time when the female managed to escape. (In many of these cases the male did not manage to insert his aedeagus.) This means that the time cost for a

TABLE 2. Differences in the behaviour of females towards 'have' and 'have-not' males

	'Haves'	'Haves' on carcass	'Have-nots'
Proportion of females accepting the male's first attempt to mate	3% (n = 32)	100% (n = 10)	88% (n = 61)
Proportion of successful first mating attempts	22% (n = 32)	100% (n = 10)	92% (n = 64)
Duration of first copulation: mean ± s.d.	25 ± 8 s (n = 27)	50 ± 13 s (n = 10)	54 ± 18 s (n = 60)

10 of the 32 'have' males were supplied with their carcass after an attracted female had stayed for 40 minutes or more: they are then called 'haves on carcass'.

female that succeeds in rejecting a copulation attempt is — on average — only 17 seconds.

The tendency of females to avoid mating with 'have' males also shows in some other data (Table 2). Even if the male succeeds in inserting his aedeagus, female reluctance can shorten the duration of the copulation. Copulations with 'have' males were significantly shorter than copulations with 'have-not' males (U-test, $p<0.001$).

However, after detection of the carcass itself, none of the females (n = 10) tried to resist copulation attempts of the male any more (Table 2, 'haves' on carcass). These matings were not significantly shorter or longer than matings with 'have-not' males (U-test, $p>0.5$).

3. Duration of stay of females and resumption of pheromone emission by the male.

Another difference in the behaviour of females meeting males of the two treatment groups concerns the females' duration of stay (Fig. 1). Regardless of their rejection or acceptance of copulations, most of the females (97%) attracted by 'have-not' males flew off a few minutes ($\bar{x}$ = 5:26 min, s.d. = 4:54 min, n = 66) after having met the male. The two females that did not fly off during the observation period remained inactive sitting under a lump of peat or a leaf.

The behaviour of females that had met a 'have' male (n = 32) was totally different. Most of them stayed for at least 40 minutes, only 25% flew off sooner than that ($\bar{x}$ = 15:30 min, s.d. = 13:35 min, n = 8). During their stay the females were continuously running across the arena and

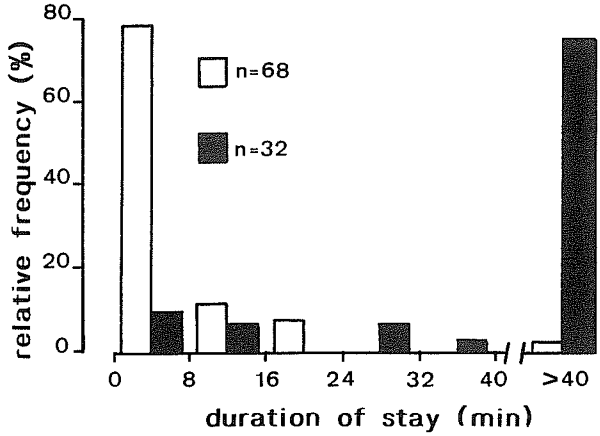

Fig. 1. Duration of stay of females after contact with the male. Females were attracted by 'have-not' males (white columns) or by 'have' males (black columns).

waving their antennae, a behaviour shown by females of both treatment groups before their first contact with the male. Some of the females who stayed for more than 40 minutes also flew off during the observation, but their flight was slow and very low above the ground, and they soon returned and continued running about.

Another difference between the two treatment groups concerns the male's behaviour. Males without a carcass usually resumed pheromone emission soon after inseminating the female. In only 15% of the observed cases did the male fail to resume pheromone emission during the observation period of that evening after having met or perceived a female. The average interval between the end of the insemination of a female and the resumption of scent emission was only 3:o5 minutes (n = 53, s.d. = 2:49 min). It has already been mentioned that 'have' males repeatedly tried to copulate with the female. Most probably this is why in 81% of the cases (n = 32) they did not resume pheromone emission during the whole evening after having met a female.

Discussion

1. Mating success of pheromone-emitting males.

The data presented here confirm our supposition (MÜLLER & EGGERT, 1987, 1988) that males who emit pheromones without having found a carcass can inseminate attracted females. They are even more successful in attracting and inseminating females than males that have buried a carcass suitable for reproduction. However, though a 'have-not' male can inseminate more females per evening than a 'have', his reproductive benefit is not correspondingly higher. Firstly, it is very uncertain that a female will find a carcass soon after having been inseminated by this male. Moreover, even if the female does find a carcass the same day and if no other male is present on this carcass, the 'have-not' male will father only a comparatively small proportion of the female's offspring. If the female's spermatheca is filled with fresh sperm from recent matings with other males, the last male to mate fathers no more than 21% of the eggs laid by the female (MÜLLER & EGGERT, 1989). If the female that has been inseminated by a 'have-not' male buries a carcass together with a second male, the 'have-not' male's sperm are even less likely to fertilize the female's eggs. However, though the expected degree of paternity is low for 'have-nots', their chances to father some offspring increase with the number of females they inseminate.

In contrast to this, a 'have' male that has found a carcass and buries it together with a female fathers a very high proportion (92%) of her offspring, because he frequently mates with the female shortly before and during the period of oviposition (MÜLLER & EGGERT, 1989).

2. Discriminating behaviour of females: when does the female distinguish 'haves' from 'have-nots'?

We have seen that females obviously can distinguish between males that have buried a carcass ('haves') and males that have not buried a carcass ('have-nots'). They accept copulation attempts of 'have-nots', but try to prevent copulation with 'haves'. If attracted females were able to tell 'haves' from 'have-not' males before having met them, we would expect them to strongly prefer 'haves' over 'have-nots', since the presence of a 'have' indicates the presence of a carcass suitable for reproduction. Our results show that the effectiveness of attraction per hour of scent emission is the same for both treatment groups. For the other measured variables concerning male and female behaviour before their first contact there are no significant differences between the two treatment groups either. We conclude that females cannot distinguish 'haves' from 'have-nots' before they come into direct physical contact with the male.

3. Adaptive value of the females' behaviour.

Most of the females attracted by 'have-nots' do not try to keep the male from copulating, although they are capable of avoiding or at least shortening copulations. Theorists have argued that females might accept multiple insemination just in order to save time, or in order to enhance the genetic variability of their offspring (WALKER, 1980; KNOWLTON & GREENWELL, 1984). Here the 'time' argument seems very unlikely, since reluctant females suffer less time cost than females accepting copulations: both the duration of copulations with reluctant females and the duration of unsuccessful mating attempts are shorter than copulations with 'willing' females. The 'genetic variability' argument is hardly testable. We suppose that the main advantage for a female that accepts insemination by a 'have-not' is the transfer of fresh sperm. In case the female finds a carcass and has to bury it on her own, she needs fertile sperm in order not to waste energy with the production of unfertilized eggs. Reproduction of females without the help of a male occurs in about 40% of the cases and the longevity of sperm stored in the spermatheca is relatively low (unpubl. data).

The benefit for a female attracted by a 'have' male is obviously a different one. The female has no need to store sperm immediately, since at least one male is present on the carcass, and will mate with her repeatedly before she lays eggs. Therefore, the female need not immediately accept copulations. However, this does not explain the reluctant behaviour of females towards 'have' males. Females tried to reject matings by a male that either signalled he had found a carcass or simply smelled of carrion, while the carcass itself was not available. We suppose that there are two naturally occurring situations that correspond to these conditions:

(1) A female meets a male that has remained alone on a carcass; the carcass is temporarily not available because the male has been emitting pheromones at some distance from the carcass (see MOSEBACH, 1936). If the female allows mating only on the carcass, her reluctant behaviour may keep the male from resuming pheromone emission, which would attract female competitors to the carcass, and make him approach the carcass.

(2) The female meets a so-called 'satellite' male (DRESSEL, 1987; DRESSEL & MÜLLER, 1988; BARTLETT, 1988) that has lost aggressive contests with another male, but still stays near the carcass to mate with the female reproducing on this carcass. If satellite males emit pheromones at some distance from the carcass, females could use the absence of the carcass as a cue to recognize a satellite. Since satellite males are the losers

of competitive interactions between males, they are most unlikely to participate in parental care. Females might 'reward' parental males for their parental investment by rejecting matings with satellite males, thereby increasing the parental males' certainty of paternity.

Summary

Males of the burying beetle *Necrophorus vespilloides* were observed during pheromone emission in the field. The males emitted pheromones either after burial of a carcass ('haves') or without having found a carcass ('have-nots'). Males of these two groups were equally effective in attracting conspecific females.

Obviously females can distinguish 'have-nots' from 'haves' as soon as they meet the male for the first time. Most of the females attracted by 'have-not' males accepted copulations, whereas females attracted by 'have' males strongly rejected mating attempts of the pheromone-emitting male. A 'have' male was allowed to mate only after the female had found the carcass. Females that had been attracted by 'have-not' males usually flew off after a few minutes, whereas the duration of stay of females with 'have' males mostly exceeded 40 minutes. After having met an attracted female, 'haves' usually did not resume pheromone emission during this evening. 'Have-not' males hardly ever failed to resume scent emission after having been interrupted by a female. The benefit of the shown behaviour of 'have' and 'have-not' males as well as of females is discussed.

References

Bartlett, J. (1987). Evidence for a sex attractant in burying beetles. — Ecol. Entomol. 12, p. 471-472.

—— (1988). Male mating success and paternal care in *Nicrophorus vespilloides* (Coleoptera: Silphidae). — Behav. Ecol. Sociobiol. 23, p. 297-303.

Dressel, J. (1987). Der Einfluß der Körpergröße und der Anwesenheit von Weibchen auf innerartliche Kämpfe der Männchen des Aaskäfers *Necrophorus vespilloides* (Coleoptera, Silphidae). — Verh. Dtsch. Zool. Ges. 80, p. 307.

—— & Müller, J. K. (1988). Ways of increasing the fitness of small and contest-loosing individuals in burying beetles (Silphidae, Coleoptera). — Verh. Dtsch. Zool. Ges. 81, p. 342.

Eggert, A.-K. & Müller, J. K. (1989). Pheromone-mediated attraction in burying beetles. — Ecol. Entomol. 14, p. 235-237.

Knowlton, N. & Greenwell, S. R. (1984). The influence of female interests. — In: Sperm competition and the evolution of animal mating systems (R. L. Smith, ed.). Academic Press, London, p. 61-84.

Mosebach, E. (1936). Aus dem Leben des Totengräbers. — Natur und Volk 66, p. 222-231.

Müller, J. K. & Eggert, A.-K. (1987). Effects of carrion-independent pheromone emission by male burying beetles (Silphidae: Necrophorus). — Ethology 76, p. 297-304.

—— & —— (1988). Biologie und Fortpflanzungsverhalten des Totengräbers *Necrophorus vespilloides*. — Sber. Ges. Naturf. Freunde Berlin (N F) 28, p. 31-43.

—— & —— (1989). Paternity assurance by "helpful" males: Adaptations to sperm competition in burying beetles. — Behav. Ecol. Sociobiol. 24, p. 245-249.

Pukowski, E. (1933). Okologische Untersuchungen an *Necrophorus* F.-Z. Morphol. Ökol. Tiere 27, p. 518-586.

Walker, W. F. (1980). Sperm utilization strategies in nonsocial insects. — Am. Nat. 115, p. 780-799.

Zusammenfassung

Im Freiland wurden sterzelnde Männchen von *Necrophorus vespilloides* beobachtet. Ein Teil der Männchen hatte einen Tag zuvor ein Aas eingegraben, der andere Teil der Männchen gab Pheromone ab, ohne ein Aas zu besitzen. Beide Typen von Männchen sind gleich attraktiv für Weibchen, wenn man die Anzahl der angelockten Weibchen in Beziehung zu der Dauer der Pheromonabgabe setzt.

Sobald das Weibchen Kontakt zum Männchen aufgenommen hat, erkennt es, ob das Männchen 'Aasbesitzer' oder 'Nichtbesitzer' ist. Die meisten Weibchen, die von einem 'Nichtbesitzer' angelockt worden sind, lassen sofort eine Kopulation zu, während die Weibchen Kopulationsversuche von 'Besitzern' abwehren. 'Besitzer'-Männchen kommen erst zu einer Kopula, wenn das angelockte Weibchen das eingegrabene Aas gefunden hat. Unabhängig von einer erfolgreichen Kopulation verlassen die Weibchen ein 'Nichtbesitzer'-Männchen nach wenigen Minuten. Die meisten Weibchen, die von einem 'Besitzer' angelockt worden sind, bleiben in der Regel länger als 40 Minuten bei dem Männchen, obwohl sie das Aas bis zu dieser Zeit nicht finden konnten. 'Nichtbesitzer'-Männchen sterzeln weiter, sobald die Weibchen abgeflogen sind, 'Aasbesitzer' fingen während dieser Untersuchung nur selten am selben Abend noch einmal an, Pheromone abzugeben.

Es wird diskutiert, welchen Nutzen Männchen und Weibchen durch ihr Verhalten haben können.