

## Older males have higher insemination success in a beetle

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**Abstract.** The hypothesis that male age affects insemination success was tested in a field study of a fungus beetle, *Bolitotherus cornutus*. In this species, male competition is important in determining the number of females with which a male attempts to copulate, but females have control over whether an attempt results in sperm transfer. Thus the proportion of a male's copulatory attempts that are accepted by females is a measure of the male's insemination success, independent of his overall access to females. This proportion was found to increase significantly as a male ages; thus, older males had higher insemination success. The insemination advantage of older males could be the result of two different mechanisms: female choice of older males or greater access of older males to highly receptive females. Neither of these mechanisms could be definitely supported or refuted based on the data collected in this study; therefore, further studies are necessary to determine whether one or both of these non-mutually exclusive mechanisms account for the older male advantage.

The effect of male age on reproductive success has been of great interest to evolutionary biologists since Trivers (1972) suggested that females should choose older males. Trivers argued that females choosing older males would tend to have longer-lived offspring, if longevity was heritable. An alternative to this hypothesis of female choice for genotypic benefit is female choice for phenotypic benefit. For example, females might choose older males because they provide superior parental care, possibly due to their increased experience (Searcy 1982). However, a mating advantage of older males may not be due to female choice at all, but could be the result of an increase in success in male competition with age. This could result in older males having better territories (Searcy & Yasukawa 1983; Alatalo et al. 1986; but see Tsubaki & Ono 1987), better position on leks (Wiley 1973), or increased success in scramble competition for mates.

In practice, it has proved very difficult to separate these alternatives, or even to demonstrate a mating advantage that is really due to age. One problem is demonstrating the effect of age itself when other characters change ontogenetically. For example, in animals with indeterminate growth, the effects of body size and growth are not easily separated (Howard 1978).

In cases where a mating advantage due to male age is found, it is rarely clear whether this advantage is due to male competition or female choice.

For example, older males mate with more females in red-winged blackbirds, *Agelaius phoeniceus*, (Yasukawa 1981), but this may be due to male competition for good territories, female choice for phenotypic benefit (experienced fathers), or female choice for males with alleles that increase lifespan (Searcy 1982; Searcy & Yasukawa 1983; Weatherhead 1984; Alatalo et al. 1986). The results presented here illustrate that even when there are no obvious confounding factors such as age-related changes in size or territory quality, it may still be difficult to distinguish male competition from female choice.

*Bolitotherus cornutus* is a tenebrionid beetle that lives throughout its life cycle on polypore shelf fungi (principally *Ganoderma applanatum*) growing on dead trees. From late May through August, females lay eggs singly on the surface of the fungi, and larvae feed by tunnelling through the interior (Liles 1956). Adults can emerge late in the summer, but most larvae overwinter and emerge as adults the next year (Liles 1956). Adults can live for several years (Pace 1967), and are active at night on the surface of the fungi, with most mating and aggressive behaviour concentrated between 2400 and 0700 hours. Oviposition, however, occurs throughout the day and night; only 10–30% of eggs are laid during the peak mating period (Conner 1987).

Male *B. cornutus* possess pronotal horns, which vary allometrically with body size (Brown & Siegfried 1983); since these beetles undergo complete metamorphosis (i.e. they are holometabolous),

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body and horn size are fixed throughout adult life. Horns are used in aggressive encounters between males, including attempts to disrupt courting couples (Pace 1967; Brown 1980; Thornhill & Alcock 1983; Conner 1987). Females lack horns and show little aggressive behaviour. Males do not defend territories, but move freely among fungi on a single tree. Migration between trees is uncommon, however, so individuals can be followed throughout their lives (Pace 1967; Conner 1988; L. Brown, personal communication).

The mating behaviour of *B. cornutus* has two stages: first, male competition for access to females, followed by female acceptance or rejection of the male. Courtship begins when a male climbs onto the back of a female and turns to face the opposite direction, placing the posterior tip of his abdomen over her head and thorax (the head-to-tail position, Brown et al. 1985). The male rubs his abdomen over the female's thorax with a rhythmic side-to-side movement (Pace 1967; Brown et al. 1985). This courtship behaviour lasts from 10 min to several hours, after which the male rapidly rotates 180 degrees (to face the same direction as the female) and attempts to copulate. The number of copulatory attempts that a male makes, a measure of a male's overall access to females, is determined at least in part by male competition (Conner 1987).

After some copulatory attempts, males disengage their genitalia and remain on the females' backs facing the same direction (head-to-head); after other attempts, males either dismount and leave the female or rotate back into the head-to-tail position of courtship (Brown et al. 1985). Since males remain in the head-to-head position for long periods (3–5 h), and this head-to-head position follows copulatory attempts that tend to be the longest in duration (Conner, unpublished data), I hypothesized that the head-to-head position represented guarding of successfully inseminated females by males. The first part of this study is a test of this hypothesis; the results show that guarding is a reliable indicator of successful sperm transfer.

The second part of this study tests the hypothesis that male age affects insemination success. Several features of the natural history of *B. cornutus* described above make it possible to study insemination success with respect to male age in this species. First, males do not defend territories or care for young, and their body size remains constant throughout adult life; thus, many of the confounding variables that complicated earlier

studies of the effect of male age on reproductive success are not present. Second, it is possible to separate insemination success (i.e. inseminations per copulatory attempt) from male access to females (i.e. number of attempts). Third, individual males can be followed throughout their lives. This study focuses on insemination success independent of access to females; male competition for access to females has been discussed elsewhere (Conner 1987, 1988).

## METHODS

### Sperm Transfer

A total of 24 male and 36 female *B. cornutus* were maintained on slices of fungi, *G. applanatum*, in two plastic boxes measuring 10 × 25 × 30 cm. The boxes were placed in an observation booth with a 16:8 h light:dark cycle (clock-shifted: dark from 0800 to 1600 hours). Dim red light (present during the dark phase) was used for observation.

Thirty copulatory attempts were observed; the females of these couples were dissected within 3 h and their reproductive tracts examined for the presence of a spermatophore. Twenty of these 30 matings were ones in which the male immediately dismounted; in the other 10 matings, males remained in the head-to-head guarding position. In addition, 12 females that were guarded by males in the field were collected and frozen within 3 h and subsequently dissected.

### Field Observations

The study population was located on a dead tree with 15 living fungi (*G. applanatum*). Beetles were collected when they were first seen and taken to the laboratory for marking. Every beetle in the population was marked.

The beetles were censused from one to three times per night on a total of 171 nights. Censuses were performed on 35 nights from 22 July to 21 September 1983, 77 nights between 19 May and 28 August 1984, and 59 nights between 16 May and 27 August 1985. Since the summer of 1985 was colder than 1984 (mean nightly low temperature 10.9°C versus 12.3°C,  $t = 2.1$ ,  $P < 0.025$ ) and beetle activity was sharply reduced below 10°C, the beetles were observed on fewer nights in 1985. Observations were conducted on about 90% of the nights on which weather conditions were suitable for beetle activity.

To estimate insemination success, the population was observed for approximately 1050 h on 149 nights: 13 nights in the last 3 weeks of August 1983 and throughout the 1984 and 1985 mating seasons (the same nights on which censuses were conducted in those 2 years; see above). Most observations were made during the time when the beetles were most active, between 1200 and 0700 hours. A headlamp, equipped with an infrared filter (Kodak Wratten no. 89B) that transmits some far-red visible light, was used for illumination. The beetles remained active all night under illumination from this light, in contrast to the complete disruption of beetle behaviour that was caused by a less filtered red light. All copulatory attempts and guarding behaviours were recorded.

Male insemination success in relation to age was studied using the following procedure. The proportion of a male's copulatory attempts that were accepted by females was used as a measure of the male's insemination success independent of his access to females. Using a self-paired design, I compared this proportion in the first 30 days of a male's reproductive life to the proportion in the second 30 days of the same male's life. Thus each male's success when he was young was compared with the same male's success when he was older, controlling for male characteristics that do not vary with age. The self-paired design meant that even if the true age of a male was not estimated accurately, the changes in male success with age were still being measured. However, it is likely that the beetles were first seen close to the beginning of their adult lives (Conner 1988).

The 'reproductive age' of a beetle was estimated as the number of nights since the first sighting of a beetle, excluding nights on which observations were not conducted. Since the beetles were observed on most nights when beetles were active, this is an estimate of relative reproductive age. Reproductive age is not an estimate of the beetles' true age, because nights when the beetles were not active were not included; since these beetles often overwinter as adults (Pace 1967), winters were also not included. Insemination success was measured for 60 days because this was the approximate mean 'reproductive lifespan' in this population (Conner 1988); the actual mean adult lifespan was about a year, with some individuals living for at least 3 years (Conner, unpublished data). Reproductive age was used so that male insemination success could be compared during two 30-day time periods

when the male had approximately equal opportunity to mate. This is why nights when little or no mating activity occurred were not included.

There were 40 males in this study whose approximate adult emergence date was known and whose reproductive lifespan was at least 60 days; of these, 34 attempted at least one copulation in both the first and second 30 days of reproductive life. Only these 34 males were included in the analysis of insemination success, since inclusion of males who did not attempt would confound access to females (number of attempts) with insemination success.

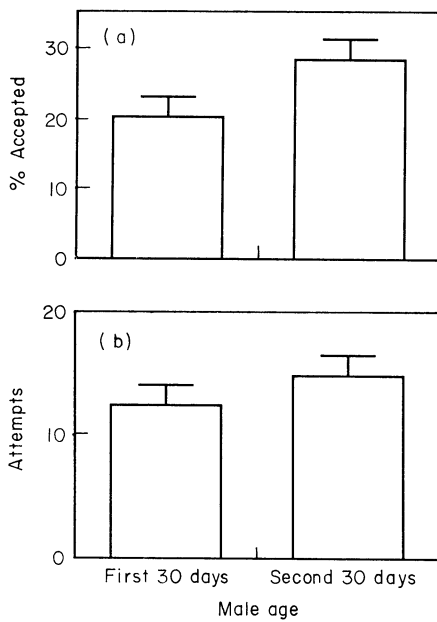
To test the effect of a female's age on her receptivity, a similar analysis was conducted. There were 45 females in this study whose approximate adult emergence date was known and whose reproductive lifespan was at least 60 days; of these, 40 received at least one copulation attempt in both the first and second 30 days of reproductive life. The proportion of all copulatory attempts that were accepted by these 40 females in the first 30 days of their lives was compared with the proportion accepted in the second 30 days.

## RESULTS

### Sperm Transfer

Spermatophores were found in all 22 females (10 laboratory, 12 field) that were dissected after males had guarded them. No spermatophores were found in the 20 females that were not guarded. Therefore, guarding is a reliable indicator that a male has transferred sperm, and an observer can determine when insemination has occurred in the field.

Dissections and observations showed that female cooperation was necessary for intromission to occur. Females have a heavily sclerotized terminal abdominal sternite that must be lowered to permit access to the reproductive tract; males have no genital hooks or claspers, and thus are unable to force entry. Unsuccessful copulatory attempts consisted of males probing at the females' closed sternite with their everted aedeagus (copulatory organ) for 3–4 min before giving up. An unsuccessful male either resumed courting the female or walked away. In a successful spermatophore transfer, females lowered the sternite, and males inserted the aedeagus for over 8 min. Therefore, whether a copulatory attempt results in sperm transfer is determined solely by the female; male



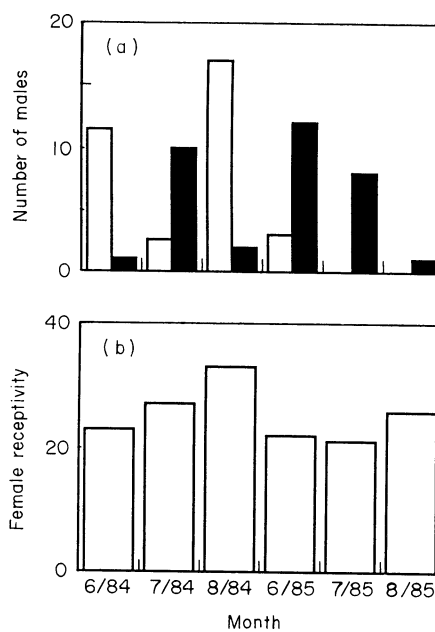
**Figure 1.** (a) Mean percentage of male copulatory attempts ( $\pm 1$  SE) that were accepted by females in the first versus second 30 days of the males' reproductive lives. Only the 34 males who lived for 60 days and had at least one attempt in both the first and second 30 days are included here. The difference is significant by the Wilcoxon signed-ranks test ( $Z=2.55$ ,  $P=0.01$ ; Snedecor & Cochran 1967). (b) Mean total number of copulations ( $\pm 1$  SE) that were attempted by the 34 males in Fig. 1a in the first versus second 30 days of life. The difference is not significant by the Wilcoxon signed-ranks test ( $Z=1.55$ ,  $P=0.12$ ).

sperm is accepted by females in about 25% of all copulatory attempts in this population (Conner 1988).

#### Field Observations

Comparison of the insemination success of individual males at different ages shows that males have greater success when they are older; females accepted a higher proportion of a male's copulatory attempts in the second 30 days of his life than in the first 30 days (Fig. 1a). This is in contrast to the comparison of total number of attempts for these same males, which shows no significant change with age in access to females (Fig. 1b).

A potential problem with this finding is that it is based on a non-random sample of the population, since only those males whose reproductive life-



**Figure 2.** (a) Frequency distribution of the month in which the majority of each male's first 30 days of life (□) and second 30 days (■) occurred. The 34 males from Fig. 1a are included here. The bars for June also include the last 2 weeks of May in each year. (b) Overall female receptivity by month. The Y-axis represents the mean percentage of all copulatory attempts that were accepted by all females in each month. These percentages are based on a total of 3208 attempts observed. The bars for June also include the last 2 weeks of May in each year.

spans were 60 days or greater were included. However, there is no evidence that males that died at a younger age differed in insemination success from those living longer. There were 17 males whose reproductive lifespans were between 30 and 60 days in this population; of these, 15 attempted to copulate with at least one female during the first 30 days of reproductive life. The insemination success in the first 30 days of reproductive life did not differ significantly between these 15 males (21% of attempts accepted) and the 34 males whose reproductive lifespans were 60 days or greater (20% accepted; unpaired  $t=0.192$ ,  $P>0.8$ ).

The increased insemination success of older males could be artefactual if female receptivity is consistently higher at some time during the mating season, and this higher receptivity coincides with the period when most males are older. To determine the seasonal distribution of the males' repro-

ductive lives, each of the two 30-day periods of each male's life was assigned to the month in which the majority of that period fell. For example, if a male was first seen in late July, the first 30 days of his life was considered to be in August and the second 30 days in June of the next year. If a 30-day period was within 1 day of being evenly split between 2 months (i.e. 14 or 15 days in one month, and 16 or 15 days in the other), then half that period was assigned to each of the 2 months. Since the peak of adult emergence is in late July and August, most of the 40 males were in their first 30 days of life in August or the following June and their second 30 days in June or July (Fig. 2a). Females are somewhat more receptive in August than in May through July (Fig. 2b). Thus the patterns of overall female receptivity and male age-structure are biased against finding an insemination advantage for older males.

If female receptivity increases with female age, and older males tend to mate with older females (positive assortative mating by age), then this could explain the finding of increased insemination success for older males. However, female receptivity declined slightly with age (28% of attempts accepted in the first 30 days of reproductive life versus 24% accepted in the second 30 days), but this difference is not significant by the Wilcoxon signed-ranks test ( $P > 0.3$ ).

## DISCUSSION

The results presented here show that male *B. cornutus* have a higher rate of insemination success when they are older. This could be due either to a real advantage of older males, or because the males in the younger age class (first 30 days) were not yet mature adults. While the latter explanation cannot be definitely ruled out, several pieces of evidence suggest that the males were mature in the first 30 days of their life. First, after emerging from the pupa, *B. cornutus* adults remain inside the pupal chamber for at least the 4 days that it takes for the exoskeleton to be fully melanized (Liles 1956). Thus, the first appearance of a beetle on the surface of the fungus (the beginning of the first 30 days in this study) occurs well after pupal eclosion. Indeed, there is no apparent reason for a male to come out on the surface of the fungi and expose himself to possible predation unless he is ready to mate. Second, the fact that there was no difference in the number of copulatory attempts in the first and

second 30 days of life suggests that males were mature in the first 30 days. Last, the complete adult lifespans of a third of the males at this site were 30 days or less, indicating that 30 days is a substantial portion of the adult lifespan of *B. cornutus*.

The insemination advantage of older males could be interpreted as evidence for female choice of older males, since insemination success is determined by the female and is independent from male competition for access to females. However, the chance that a female would accept a male's sperm (i.e. her overall receptivity) can be affected by factors that have nothing to do with the male (e.g. the female's reproductive status). If males can identify which females are most likely to be receptive, then the insemination advantage of older males could result from an increase with age in the ability to detect or compete for access to receptive females. Females are most receptive immediately after laying an egg; in fact, they are almost twice as likely to accept a copulation attempt that followed an oviposition than all other attempts (Conner 1987). Therefore, if there is an increase with age in the proportion of a male's copulatory attempts that are with these highly receptive females, then this could explain the older males' insemination advantage. A non-significant trend in this direction was found: as males aged, the proportion of their copulatory attempts that were with females that had just oviposited increased ( $0.033 \pm 0.012$  in first 30 days versus  $0.045 \pm 0.009$  in second 30 days, mean proportion accepted  $\pm$  SE of the mean;  $P < 0.2$  by Wilcoxon signed-ranks test;  $N = 34$ ). Thus, older males may have increased access to receptive females, but further study is necessary to test this hypothesis.

If older males do have increased access to receptive females, it suggests that males can recognize receptive females. Males of one species of poeciliid fish, *Poecilia latipinna*, have also been shown to give increased numbers of courtship displays to highly receptive females (Farr & Travis 1986). It is not clear in either *P. latipinna* or *B. cornutus* how males recognize receptive females, although the courtship behaviour of both species suggests that chemical communication may be occurring (Brown et al. 1985; Farr & Travis 1986, personal observation).

It is unclear how older males would gain greater access to receptive females than younger males. It could be that older males are better able to recognize receptive females, or are better able to

compete for access to them, or both. Other studies that have addressed the question of the effect of male age on mating success have suggested that age affects male competitive ability (Campanella & Wolf 1974; Woodhead 1986; Tsubaki & Ono 1987). All these studies were on insects that do not grow as adults. Thus the increase in competitive ability with age in these insects and in *B. cornutus*, if it does exist, would have to be caused by some mechanism other than a size increase. One possibility is increased experience in aggressive encounters, as suggested by Campanella & Wolf (1974).

The higher insemination success of older males may also be due in part to female choice of older males. Indeed, even 'highly receptive' female *B. cornutus* reject over half of the males that attempt to copulate with them (Conner 1987). This might suggest that female choice is occurring; however, this could also mean that females are only receptive about half the time after they have laid an egg. The concept of receptivity is a difficult one here, since the only way to determine if a female is receptive is to see if she accepts a male; if she does, then there is no way to tell if she was choosing that specific male or was just generally receptive.

To demonstrate female choice, one has to show that females reject some males and accept others (Halliday 1983). Because only one male can court a female at a time in *B. cornutus*, female choice in this species could consist of females rejecting the first male that attempts to copulate, and then accepting another male's sperm later in the same night. This was observed 63 times in this study. In 44 of these cases the ages of the two males involved were known to be different; the accepted male was older in 27 cases (61%;  $G=2.29$ ,  $P<0.15$ ). While this trend is suggestive, controlled experiments are necessary to definitively test the hypothesis of female choice for older males. For example, characteristics other than age of the males should be controlled, as should the order in which the males are presented to the female. In fact, in the 44 cases above, the accepted male was heavier in 26 (59%;  $G=1.46$ ,  $P<0.25$ ). Thus the differences in weight could account for much of the trend for older males being chosen by females. However, in laboratory experiments addition of weights to males did not affect their rate of acceptance by females (Conner, unpublished data). There were no differences in horn or elytra length between accepted and rejected males.

It is unlikely that female *B. cornutus* could

choose males based on age itself, since this would require that females know the ages of males. However, females might select males based upon some character that is highly correlated with age. An obvious possibility is that some aspect of male courtship behaviour changes with experience (as was suggested above for aggressive behaviour). Kruijt et al. (1972) suggested that experience might increase the courtship effectiveness of male black grouse, but to my knowledge there is no evidence for changes in courtship behaviour with age in any species.

In conclusion, male *B. cornutus* have greater insemination success when they are older. It is not clear, however, whether this advantage is caused by female choice of older males, increased male access to highly receptive females, or perhaps a combination of the two.

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