

Male reproductive incompetence, fertility, and the cost of mating in male seaweed flies

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ABSTRACT

Male seaweed flies were paired with a succession of virgin females, and the total number of progeny sired by each male was determined. The life-time fertility of males is shown to be about four-fold higher than that of females. Not only are males able to mate repeatedly, but in the short term they remain able to fertilize many females. However, old males exhibit a decline in reproductive competence. This is shown to result from a reduction in the mount rate and in an increased rate of dismounting by the male. No evidence was obtained that the supply of sperm had become exhausted.

The change in the weight of males was measured throughout their lifetime. No change was observed that was attributable to sexual activity, though a significant shortening of life expectancy occurred. There appears to be no substantial cost of reproduction to males.

The results are considered in the context of reproductive investment, and its

relevance to the evolution of male mate choice is discussed.

INTRODUCTION

Organisms have access to a finite pool of resources with which to survive and reproduce. If resources used in reproduction diminish those available for survival or future fertility (Williams 1966), there is a so-called 'cost of reproduction' (Reznick 1985; Bell and Koufopanou 1986). It has been readily accepted that such a cost exists in females. They produce nutrient-rich eggs, and many expend energy in mating and parental care (see Partridge et al. 1987; Fowler and Partridge 1989). For some time it was thought that males invest substantially less in reproduction than do females, and students were often taught that males produce vast numbers of cheap sperm, mate repeatedly and usually indiscriminately, and continue making sperm for most of their adult life. This naive view of male reproduction is now changing in the light of evidence that male investment in reproduction is often far from negligible.

During mating sperm are never transferred in isolation; the costs of the

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other seminal components such as accessory gland fluid may far outweigh those involved in the production of the sperm themselves (Dewsbury 1982). Perhaps the most extreme examples of male reproductive costliness, are those insect species that transfer large spermatophores during copulation; they include Mecoptera (Thornhill 1976), Lepidoptera (Boggs and Watt 1981; Drummond 1984), Neuroptera (Hayashi 1993) and many Orthoptera species (Bowen *et al.* 1984; Gwynne 1984, 1990; Gwynne and Simmons 1990). It is often the case that mating declines in the period of recovery from depletion of the accessory glands (reviewed by Drummond 1984), and males may even become temporarily reproductively incompetent after mating repeatedly (Freund 1963; Nadel and Luck 1985; see also review by Dewsbury 1982). Permanent, senile reproductive incompetence may also occur (Lefevre and Jonsson 1962; Jones 1973; Simmons 1988).

Such constraints on the ability of males to reproduce have profound consequences on the evolution of male mating behaviour. Trivers (1972) suggested that the strength of mate choice is a consequence of the investment in reproduction, and offered this as an explanation for the fact that females are usually more discriminating in their choice of mate. However, if males invest substantially in reproduction we should expect them to exhibit strong mate choice. There is now considerable evidence that males as well as females can exercise mate choice (Manning 1975; Berven 1981; Gwynne 1981; Van den Burghe and Warner 1989; Cote and Hunte 1989; Gwynne and Simmons 1990).

Here we report a study of the constraints that may exist on male reproductive performance, in an attempt to

promote understanding of male mating behaviour and the genetical consequences of male mate choice.

MATERIALS AND METHODS

The animals

The flies originated from a natural population at St. Mary's Island on the north-east coast of England. A programme of laboratory crosses was undertaken to obtain the SMI-C strain, a line homozygous for the C allele at the alcohol dehydrogenase locus. Details of the derivation and routine maintenance are described in Pitafi *et al.* (1990).

Sequential mating experiment

Virgin adults were collected and stored at 4°C with cotton wool soaked in 0.5% solution of mannitol. (This sugar, present in high concentrations in brown seaweeds, is used by adults as a food source.) The sexes were kept separate. When required the females were kept on mannitol, but transferred to 26°C. The males were transferred onto freshly minced seaweed (*Fucus serratus* and *F. vesiculosus*) at 26°C for 48 h. This period of sex deprivation in the presence of food resulted in the males being very willing to mate when presented with a potential mate who had not previously laid eggs.

Single males were placed in a plastic arena (10mm x 50mm diam.) containing minced seaweed. When a female was introduced, a mount usually occurred within a few seconds. Immediately the male dismounted, the two animals were removed, the male replaced, and a new virgin female introduced to the arena - all transfers being carried out without anaesthesia. If no copulation took place within 15 min, the female was replaced with another female. This sequential replacement of females was continued for 2.5h or until the male failed to mate with

three consecutive females. The male was then transferred to larger pots (70mm x 75mm diam.) containing minced weed and 10 virgin females, and left overnight. The following morning each male was observed with another sequence of single females. This routine of sequential presentation of single females during the day alternating with 10 females available overnight was continued until the male died.

Every female, whether day-time or night-time, was placed individually into a small pot (40mm x 45mm diam.) containing weed and kept at 26°C. Pots were checked daily for eggs, and if present, the female was transferred to a new pot. Daily checking and transferring when necessary, were continued until the females died. Pots containing eggs were kept for a further two days after which time all egg cases, as well as unhatched eggs, were stored in test tubes containing methanol for subsequent counting. Three categories of eggs were distinguished: egg cases from which larvae had hatched, unhatched eggs in which a larva was clearly visible (embryonic lethal genes are not uncommon in *C. frigida* - Burnet 1961, 1962) and finally, eggs that had not been fertilized at all. The first two categories were summed to determine the number of eggs fertilized. Females lay their eggs in clutches at roughly two day intervals. The third and subsequent clutches included few fertilized eggs but were nevertheless collected and examined to determine the total number of fertilized eggs laid by each female. Because of the labour-intensive nature of this experiment only five males were analysed thoroughly. For a further seven males, all the females were scored as producing fertilized eggs or not, but the number of fertile eggs was not determined.

Is there a detectable cost to males of mating?

Virgin adults were used without prior storage at low temperature. Pots containing minced weed were set up with either two males, one male and one female, or five males and one female. The sizes of all adults were determined at the start of the experiment by measuring their wing lengths (Butlin et al. 1982). In the two-male trials the adults were uniquely identified by clipping the end of one wing, a procedure that has been shown not to influence mating (Day et al. 1990). All adults were weighed individually each day using a Mettler H20 balance accurate to 10µg, except for trials involving five males; in these trials the total weight of the males was measured. This regime of daily weighings was continued until either the female died, or all the males had died. At least 11 replicates of each trial were performed.

RESULTS

Do males become reproductively incompetent?

Reproductive incompetence in males may arise for two reasons. Temporary, or short-term incompetence, may result when the reserve of semen is depleted but, given food and time, the necessary substances can be replenished. Alternatively, there may occur a permanent loss of ability to synthesize seminal components or any other chemicals required for mating. In this case there would exist a post-reproductive phase in the life cycle.

Whether the sterility is temporary or permanent it may manifest as a behavioural impotence - an unwillingness or apparent disinterest in mating, and then it could be very difficult to demonstrate that its underlying cause is exhaustion of semen.

Let us consider short-term incompetence first. Single virgin females were made available to individual males and their mating behaviour observed. Following each mating, the male was presented with a new female. This sequential presentation of females was continued for 2.5 h on the first day. The five males analysed mounted 9, 10, 14, 16 and 35 females respectively. Males are clearly able to mate repeatedly, and without exception, appeared still to be willing to mate after the 150 min of the trial. One male was observed for over 250 min (during which time he had mated with 35 females), and he still retained his willingness to mate. Summing over all males, 61.5% of the mated females subsequently produced progeny. The fact that almost 40% of females were not fertilized, admits the possibility that the males were becoming exhausted of semen. However, the number of females fertilized in the latter halves of the sequences (a total of 68 out of 107 females presented) was in fact slightly higher than in the first half (62/107). The difference was not significant (heterogeneity $\chi^2 = 0.71$, $df = 1$, $p = 0.40$). Another possibility is that as the sequence progressed the females received a diminishing number of sperm. This would predict a correlation between the number of eggs fertilized and the sequence order of the female. None of the sequences showed such a correlation (Table 1). We conclude that the somewhat low fertilization rate (about 60%) is not a consequence of the male becoming depleted of semen after repeated matings.

Consider next long-term reproductive incompetence. The sequential presentation of females was continued during the daytime until the male died. The mean longevity of 12 males was 4.3 d. Between daytime trials each male was allowed access to 10 virgin females. The fertility of all females was assessed, but

Table 1. Relationship between the number of eggs fertilized, and the ordinal number (1st, 2nd, 3rd etc.) of the females mated in sequence. N = number of females in each sequence; R = Spearman's rank correlation coefficient; P = probability. Combined probabilities were obtained by the method of Fisher (1934).

Male	N	R	P
1	23	- 0.09	0.68
2	6	+ 0.20	0.65
3	11	+ 0.30	0.34
4	9	- 0.43	0.22
5	5	+ 0.70	0.16
Combined probability: $\chi^2 = 2.88$, $df = 10$, $p = 0.98$			

the males' behaviour was only observed with the daytime females. The results are given in Table 2; differences referred to in the following section are all statistically highly significant ($p < 0.005$).

With approaching death the mount rate declined, but even at its minimum, 60% of the females were mounted. The rate of dismounting rose dramatically from virtually zero to 85%. The combined effects of mounting and dismounting - both of them exclusively male actions - was that the proportion of females mated dropped from over 97% to less than 10%. We interpret these changes as resulting from a reluctance on the part of the male to proceed with the mating process.

Focussing on the females that were mated, a striking reduction in the rate of

Table 2 Mount rate, dismount rate, and percentages of productive and unproductive prolonged mounts on females provided in succession to males throughout their lives

Day	Male number (size - mm)												
	1 (5.7)	2 (5.6)	3 (4.5)	4 (5.7)	5 (4.2)	6 (5.6)	7 (5.7)	8 (4.0)	9 (3.9)	10 (3.9)	11 (4.1)	12 (4.5)	
1	N	35	10	17	15	9	16	15	4	11	5	10	5
	M	100	100	100	100	100	100	100	100	100	100	100	100
	D	0	0	0	0	0	0	0	0	0	20	0	60
	PM, no L	34	40	35	40	44	25	20	0	55	80	60	40
	L	66	60	65	60	56	75	80	100	45	0	40	0
2	N	4	4	7	6	2	3	10	2	5	5	4	3
	M	0	0	100	100	100	100	100	100	100	100	100	100
	D	0	0	14	0	100	0	0	100	20	80	50	0
	PM, no L	0	0	0	83	0	67	10	0	20	20	50	100
	L	0	0	86	17	0	33	90	0	60	0	0	0
3	N	4	4	4	4	9	3	7	†	2	2	2	2
	M	0	0	75	75	100	100	100		100	100	100	100
	D	0	0	50	0	0	33	29		100	100	100	0
	PM, no L	0	0	0	50	44	33	14		0	0	0	100
	L	0	0	25	25	56	33	57		0	0	0	0
4	N	†	4	2	2	5	4	4		2	2	†	5
	M		0	50	50	100	100	100		100	50		100
	D		0	50	50	0	25	25		100	50		40
	PM, no L		0	0	0	60	25	25		0	0		60
	L		0	0	0	40	50	50		0	0		0
5	N		†	4	†	4	2	2		†	2		†
	M			100		100	50	100			100		
	D			100		50	50	0			100		
	PM, no L			0		25	0	0			0		
	L			0		25	0	0			0		
6	N			†		2	2	2			†		
	M					100	100	0					
	D					100	0	0					
	PM, no L					0	50	0					
	L					0	50	0					
7	N					†	2	†					
	M						0						
	D						0						
	PM, no L						0						
	L						0						

N = No. of females provided each day
 M = Percentage of N that were mounted
 D = Percentage of mounted females that were dismounted
 PM, no L = Percentage of mounted females that were prolonged mounted, but subsequently produced no larvae
 L = Percentage of mounted females that were prolonged mounted and also produced larvae
 † = Male had died overnight

fertilization was observed. By two days before the males' death the fertilization rate had almost halved (31.3%), and after a further day none of the three mated females produced progeny. This suggests that in spite of the male completing the behavioural components of mating, competence to transfer effective sperm was seriously impaired. A more reliable view of competence is obtained when both day- and night-time females are included in the analysis. The overall rate of fertilization of available females strongly declined with approaching death. Of the 153 females available to males one day from death, only 8 produced progeny, and in fact, 4 of these were mated by the same male.

These results indicate that males do become reproductively incompetent, partly as a result of a reluctance to remain mounted on females, and partly because of a diminished ability to transfer effective sperm. It is possible that the change in dismount rate is itself triggered by the exhaustion of semen.

Estimation of male fertility

The experiment described above was not primarily designed to yield information on male fertility. However, since five individual males were throughout their life in the presence of an excess of virgin females, and all the fertilized eggs laid by those females were counted, an estimate of male fertility can be obtained. The mean number of progeny (including fertilized eggs that died during embryogenesis) was 2284 (st. err. = 292) with a range of 1483 - 2957. These results can be compared with those of Brundell (1988) who used a rather different method. He allowed single virgin males continuous access to 100 virgin females and then counted all the adult progeny produced. He used flies collected the previous generation from a population at St. Mary's Island (the same

population from which the SMI-C stock was derived). Although excess food was provided for the progeny larvae, it seems unlikely that all fertilized eggs survived to adulthood. The mean male fecundity based on 12 males was 1913 (st. err. = 366) with a range of 425 - 3750. Considering the differences in the animals and in the experimental design, these estimates are surprisingly similar ($U = 23$, $z = 0.74$, $p = 0.46$).

Is there a cost to males of mating?

The most extreme form of reproductive investment is seen in those species that transfer a nutritionally rich spermatophore (see Introduction). In other species the females gain little from the male apart from sperm, but the male nevertheless expends substantial energy in the mating process, perhaps while engaged in male-male competition. When this happens the adult life-span of the male is often shorter when he is sexually active. This section is concerned with whether there are detectable differences in weight loss and in the longevity of male *C. frigida* associated with mating.

The males were either in the presence of another male (12 trials), or with a female (12 trials), or with a female and four other males (11 trials). Males were weighed daily throughout their life and their wing lengths also measured. The weight of each male was expressed relative to its weight one day after eclosion. No correlation was found between wing length and either longevity or mean daily weight change - again probably because the males used were chosen to be of similar size. The analyses have therefore been carried out with no further regard to body size.

The mean longevities (\pm st. err.) of mated males were as follows: one male with female = 9.83 (0.64) d; five males

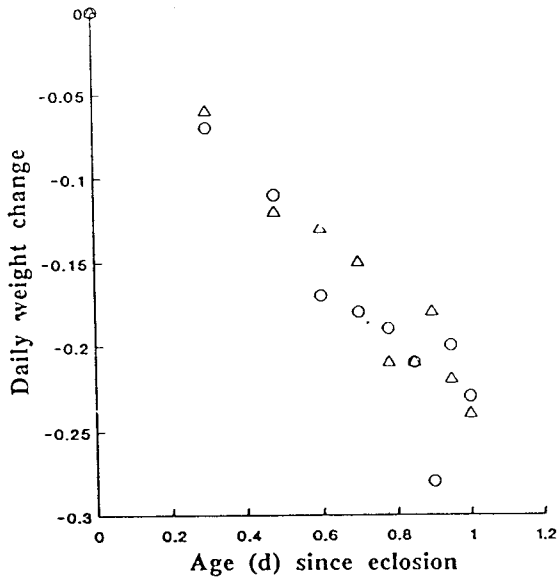


Figure 1 Comparison of weight changes in mated males in the presence and absence of other males (ie. with and without male-male competition). Both weight change and age are transformed to logarithms. Triangular symbols refer to trials with one female and one male; circular symbols refer to trials with one female and five males.

with female = 9.89 (0.43) d. Since the presence of other males does not influence longevity ($t = 0.06$, d.f. = 65, $p = 0.95$), there is no evidence that male-male competition has any cost in terms of the life-expectancy of males. The mean longevity of unmated males was 11.25 (0.43) d, a value that is significantly greater than that for mating males (pooling the data for all males in the presence of a female, and using Bailey's correction for unequal variances (Bailey 1981): $t = 2.44$, d.f. = 58.2, $p = 0.017$). This result suggests that there is a cost to mating in that sexual activity shortens the life expectancy of males by about 13%. This is consistent with findings from other species of insects (see Ridley 1988).

Consider next the weight changes observed in males. The weights of males (relative to their eclosion weights) were plotted against time (with both variables transformed to logarithms) for all three categories of males (Figures 1 and 2). Both the slopes and the intercepts of these lines were remarkably similar. Comparing the slopes of mating males with and without other males present (Figure 1): $t = 0.065$, d.f. = 13, $p = 0.95$, and comparing the intercepts of these lines : $z = 0.066$, $p = 0.95$. As with longevities, there appears to be no cost associated with male-male competition. Comparing the slopes of unmated and mating males (Figure 2): $t = 0.11$, d.f. = 15, $p = 0.91$, and comparing their intercepts: $z = 0.113$, $p = 0.91$. There appears to be no

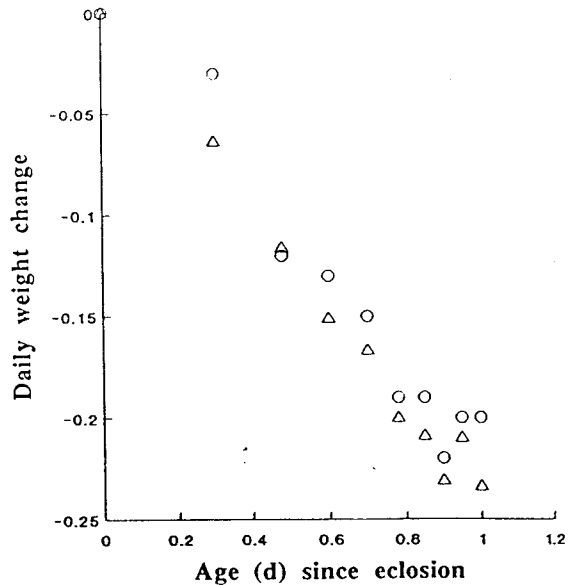


Figure 2 Comparison of weight changes in mated and unmated males. Both weight change and age are transformed to logarithms. Triangular symbols refer to trials with one female and at least one male; circular symbols refer to trials with two males.

detectable cost associated with mating in terms of weight change of males. We interpret this to mean either that there is a trivial expenditure of energy in mounting and copulation, or that the energy consumed is roughly balanced by extra feeding. Unfortunately it is difficult to distinguish these alternatives since males rarely mate in the absence of food.

Analyses of the longevities, weight changes and fecundities of the females rather than males, yielded no evidence for the transfer of nutrients from the males during copulation. The justification for this conclusion will be reported in detail elsewhere.

DISCUSSION

In many mammals and insects, repeated ejaculations result in males becoming temporarily depleted of sperm or other seminal components (see references cited in Introduction). In contrast, male seaweed flies are able to fertilize a long succession of females within a short time span. It proved difficult to obtain convincing evidence that any short-term depletion occurs. Presumably either the seminal reserves are exceptionally large in *Coelopa*, or very little semen is transferred during each copulation. In their natural habitat adults are attracted to hot spots in decomposing seaweed deposits (Burnet 1961). Thousands, or even hundreds of thousands, of adults may collect so that the encounter rate between them must be very high indeed. It is also known that females remate many times, probably rarely with the same male, before they oviposit, and that in consequence there exists the opportunity for intense sperm competition. Given such a pattern of extreme polygamy and explosive breeding (*sensu* Alcock 1989) it is to be expected that males retain reproductive competence following many inseminations in quick

succession. Following such intense reproductive activity it is perhaps not surprising that senile males exhibit a reduced mount rate and an increase in the dismount rate. Even after those few mounts by old males that do proceed to prolonged genital engagement, relatively few eggs are actually fertilized. Whether the behavioural disinterest in mating is a result of sperm depletion remains obscure, but the consequence is clear - males can and do become reproductively incompetent before they die.

Is this of any consequence in natural populations? If males normally die before they become impotent, their impotence under laboratory conditions is an irrelevance. However, predation and parasitism are not thought to be major causes of mortality in seaweed flies (Leggett 1993); the only known predators of *C. frigida* are a few species of shore birds (Cullen *et al.* 1987), and these have a negligible impact on the vast population sizes of adult flies, which often exceed 10^6 (Butlin and Day 1989). It is therefore likely that senile males do remain in the adult population; certainly the adult population includes senile females (Hewitt, unpublished results). If this is so, we should expect mate choice to evolve with females discriminating against impotent males. Female mate choice occurs in *C. frigida* with respect to male size (Gilburn *et al.* 1992), but there has been no study of whether they also prefer young males.

The results presented here show that the energetic costs of mating in *C. frigida* are not sufficiently substantial to result in sexually active males losing more weight. Nevertheless, like very many other insects (see Ridley 1988), their life expectancy is reduced. This points towards there being some cost, albeit rather modest, arising from the physical activity of mounting,

copulating and dismounting. There is no evidence of energy-consuming fights with other males, nor of the transfer of detectable quantities of nutrients to the female.

While not the primary objective of this study, estimates of male fertility were obtained that are worthy of comment. There are very few species in which the life-time reproductive success of males has been estimated, and even fewer (if any) in which it can be compared with female fertility. The mean life-time fertility of females from the strain used in this study was 562 (Pitafi and Day unpubl. results), whereas the equivalent figure for males was 2284 - a four-fold differential between the two sexes. This does not seem to us to be a gross discrepancy, and it certainly does not suggest that male insects have a very much greater reproductive potential than do females.

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