Behav Ecol Sociobiol (2005) 58: 128–135
DOI 10.1007/s00265-005-0922-x

ORIGINAL ARTICLE

D. W. Dunn · J. P. Sumner · D. Goulson

The benefits of multiple mating to female seaweed flies, *Coelopa frigida* (Diptera: Coelpidae)

Received: 13 August 2004 / Revised: 30 November 2004 / Accepted: 25 January 2005 / Published online: 9 March 2005

Abstract Female seaweed flies, *Coelopa frigida*, have the potential to benefit from mating more than once. Single matings result in low fertility so females may benefit directly from multiple copulations by sperm replenishment. A chromosomal inversion associated with larval fitness, with heterokaryotypic larvae having higher viability than homokaryotypes, means that polyandrous homokaryotypic females have a higher probability of producing genetically fit offspring than monandrous homokaryotypic females. We allowed females to mate only once, repeatedly four times to the same male, or polyandrously four times to four different males. Multiply mated and polyandrous females laid more eggs and produced more offspring than singly mated and monandrous females, respectively. Polyandrous females laid more eggs, had higher egg-to-adult survival rates and produced more offspring than repeatedly mated females. Fertility rates did not differ between treatments. The observed fitness patterns therefore resulted from increased oviposition through multiple mating per se, and a further increase in oviposition coupled with higher egg-to-adult offspring survival benefits to polyandry. Daily monitoring of individual females over their entire life spans showed that multiple copulations induced early oviposition, with polyandrous females ovipositing earlier than repeatedly mated females. Singly mated and polyandrous females incurred a longevity cost independent of egg production, whereas repeatedly mated females did not. This suggests that repeatedly mating with the same male may counteract a general cost of mating. Longevity, however, was not correlated with overall female fitness. Our data are discussed in the overall context of the seaweed fly mating system.

Communicated by G. Wilkinson

D. W. Dunn (✉) · J. P. Sumner · D. Goulson
Ecology and Evolution Group, School of Biological Sciences,
University of Southampton,
Basset Crescent East,
Southampton, SO16 7PX, UK
e-mail: derek.dunn@soton.ac.uk
Tel.: +44-2380-594268
Fax: +44-2380-594459

Keywords Polyandry · Costs of mating · Sexual conflict · *Coelopa frigida*

Introduction

The females of most animal species mate more than once, and usually do so with more than one male (reviewed in Birkhead and Møller 1998; Birkhead 2000). Although the fitness benefit to males from mating with multiple partners has been recognised since the work of Bateman (1948), the adaptive significance of this behaviour to females has remained controversial. Current opinion categorises the benefits of multiple mating to females as either material benefits, which may be available to both monandrous (by repeatedly mating with the same male) and polyandrous females, and genetic benefits (Jennions and Petrie 2000), which are only available to polyandrous females (Fedorka and Mousseau 2002). Making a clear distinction between direct and genetic benefits, however, is difficult because polyandrous females often have the potential to gain both (Jennions and Petrie 2000). Experimental approaches therefore need to distinguish between mating repeatedly with the same male and polyandry (Tregenza and Wedell 1998; Newcomer et al. 1999; Eady et al. 2000; Baker et al. 2001; Fedorka and Mousseau 2002; Zeh and Zeh 2003).

There are several material benefits available to females by mating multiply. These include obtaining material resources from males that can translate directly into increased offspring numbers (Vahed 1998; Wiklund et al. 2001), reducing the costs of male harassment (convenience polyandry: Thornhill and Alcock 1983; Rowe et al. 1994), and sperm replenishment (Jones 2001). The genetic benefits potentially available to polyandrous females include avoiding mating with a sterile male (Olsson and Shine 1997; Lorch and Chao 2003); avoiding inbreeding (Madsen et al. 1992; Stockley et al. 1993; Hosken and Blanckenhorn 1999; Tregenza and Wedell 2002) or a genetically incompatible mate (Tregenza and Wedell 1998; Newcomer et al. 1999); initiating sperm competition

(Keller and Reeve 1995; Simmons 2001) or postcopulatory mate choice (Eberhard 1996), and bet hedging (Lorch and Chao 2003) to produce genetically variable offspring in stochastically variable environments (see also Williams 1975). A recent meta-analysis of 122 papers concluded that females in the majority of insect orders obtain direct benefits of polyandry, primarily through sperm replenishment and/or nuptial gifts from males (Arnqvist and Nilsson 2000). Arnqvist and Nilsson's findings, however, also include any genetic benefits to females if present, because the effects of multiple mating with the same male and polyandry were not separated.

Female seaweed flies, *Coelopa frigida*, have the potential to benefit genetically by mating polyandrously (see Jennions and Petrie 2000). In *C. frigida*, the αβ inversion on chromosome one is strongly associated with adult body size, female mating preference for male size in some populations, and larval fitness (Wilcockson et al. 1995; Day and Gilburn 1997). Adult αα homokaryotypes are large, ββ homokaryotypes are small, and αβ heterokaryotypes are intermediate in size. There is larval heterosis with heterokaryotypic larvae having higher egg-to-adult viability than homokaryotypes (Butlin et al. 1984). Therefore, there should be strong selection for females to mate disassortatively with regards to male size and consequently inversion karyotype. Females would benefit genetically by producing highly fit heterokaryotypic offspring (Gilburn and Day 1996). However, this is not the case as female *C. frigida* usually mate with large males, probably because large males are most effective at overcoming females' reluctance to mate (Crean and Gilburn 1998; Crean et al. 2000; Shuker and Day 2001, 2002). Polyandry may enable homokaryotypic female *C. frigida* to produce genetically highly fit offspring, even if individual opportunities for precopulatory mate choice are compromised by coercive male behaviour.

There may also be direct benefits available to female *C. frigida* that mate multiply. High densities of adults congregate in and around large deposits of beached marine algae (wrackbeds), on which the larvae feed (Cullen et al. 1987). Females incur high levels of harassment (J. Blyth and A.S. Gilburn, personal communication), are reluctant to mate even as virgins (Day et al. 1990; Gilburn et al. 1992; Crean et al. 2000; Dunn et al. 2002), and become more receptive as male harassment increases (Shuker and Day 2001; see also Blanckenhorn et al. 2000). This suggests that female *C. frigida* exhibit convenience polyandry, and they may mate with some superfluous males to trade-off the costs of rejecting many males (see also Rowe et al. 1994). Moreover, a previous experiment found that the fertilisation rates of singly mated females are low-approximately 60% (Pitafi et al. 1994). A single mating may therefore be unlikely to provide enough sperm to fully enable a female to fertilise all of her eggs, and females may benefit directly by mating multiple times through sperm replenishment.

Mating has costs as well as benefits, especially for females (Daly 1978), which have to be accounted for to fully understand reproductive behaviour (Arnqvist and Nilsson 2000). Longevity costs of mating have been identified in several insect species in which females demonstrate resistance behaviour when mounted by males (e.g. Crudgington and Siva-Jothy 2000; Blanckenhorn et al. 2002). Identifying similar costs of mating to female *C. frigida* would greatly facilitate our understanding of the evolution and maintenance of female precopulatory reluctance (Crean et al. 2000; Dunn et al. 2002) and female mate choice for large males in the Coelopidae (Crean et al. 2000). Longevity costs of mating to female *C. frigida* have remained unidentified, but experimental approaches have largely centred on females that have only been mated once (Shuker 1998; Dunn et al. 2002).

The purpose of this paper is to examine some of the potential fitness benefits and longevity costs of multiple mating to female *C. frigida*. This species is clearly important for use in studies examining the adaptive significance and evolution of polyandry, because there are likely direct and indirect benefits potentially available to females. The design of our experiment will distinguish between the effects of mating repeatedly with the same male and mating an equal number of times with different males, to separate material and genetic benefits to females.

Methods

The animals used for the experiment were from an outbred laboratory stock, which originated from >500 larvae captured at Kimmeridge Bay, Dorset, UK in 2003. A large laboratory population (>1,000 individuals) was reared in the laboratory for three generations prior to the production of experimental virgin flies. Fresh seaweed (*Fucus serratus*) was used as the sole larval food source (see Day et al. 1982 for rearing methods) and was also collected from Kimmeridge Bay.

The flies used in the experiment were collected as virgins twice daily, separated by sex using a mild CO_2 anaesthetic, then stored with cotton wool soaked in 5% sucrose solution at 4°C for up to 3 days. Males were then individually placed into small (40 ml) ventilated plastic containers containing freshly minced *F. serratus* for a food and moisture source, and because algae exposure induces male willingness to mate (Dunn et al. 2002). Fifty females were collectively placed into a 500 ml glass bottle with cotton wool soaked in 5% sucrose solution as a food and moisture source. All experimental flies were then placed in a constant temperature room at 25°C with a 12:12 h light:dark cycle for 48 h.

Experimental procedure

We used four experimental treatments with 60 replicates per treatment: (1) virgin females; (2) females mated once (hereafter referred to as singly mated); (3) females mated four times to a single male (hereafter referred to as repeatedly mated), and (4) females mated four times, each time to a different male (hereafter referred to as polyandrous).

To obtain singly mated females we randomly assigned a female to an upturned culture dish (55 mm in diameter), then introduced a randomly chosen male. If the male was unwilling to mount the female within 5 min (see Dunn et al. 1999, 2002) he was removed and another male was introduced to the female until copulation occurred. Copulation was determined when the male was seen to actively engage his genitalia with that of the female. The pair was then left in copula until the male was forcibly removed by the female or until he dismounted voluntarily. The duration males remained mounted on females was timed in seconds.

To obtain repeatedly mated females, the same process as described for females mated singly was undertaken, except individual pairs were observed until they had mated and separated four times. Males would usually remount the female almost immediately. In this treatment, no male that copulated once failed to mate three additional times.

To obtain polyandrous females, for a female's first mating the same process as described for singly mated females was repeated. When the first mating had ended the male was removed from the culture dish. The female was then immediately presented with a second randomly chosen male that had previously mated once with another female. This process was repeated until the female had mated with two different additional males that had mated previously two or three times for that female's third and fourth copulation respectively. This ensured that the mating history of males for the repeatedly mated and polyandrous treatments were the same.

Randomly selected virgin control females, and all mated females, were individually placed into small (40 ml) ventilated plastic containers containing freshly minced *F. serratus*. Each fly was checked daily until death. If eggs were present, the female was transferred to a fresh container of algae, then the eggs were counted. We estimated the fertility of non-control females. This was done by carefully removing 20 randomly selected eggs on the first day eggs were present with a small, soft-bristled paint brush, then placing them into a 20 ml culture dish containing a small piece of fresh algae to prevent desiccation. The proportion of these 20 eggs that hatched was then scored 24 h later (see also Martin and Hosken 2002).

For a randomly chosen 50% (30 replicates) of each non-virgin treatment, all eggs laid by an individual female (and the first instar larvae from the same female resulting from the fertility estimate) were placed into a 250 ml ventilated plastic container filled with minced *F. serratus*. This amount of algae is sufficient for the larvae of one female to develop without resource competition (Crean 1997). Larvae were allowed to develop, pupate and eclose for a measure of lifetime reproductive success for each female. Adults were collected daily and counted from the first day of eclosion. This prevented female offspring from mating with siblings and laying eggs back into the larval medium from which they had eclosed.

All males were used for one bout of mating only except for the polyandrous treatment in which males were randomised between females as previously described. All females were only used once. The body size of each fly was estimated by measuring wing length, which has been the standard method used in most previous studies in coelopids, e.g. Crean et al. (2000). All fly and larval containers were kept in the same environmental conditions and were randomly repositioned each day to control for any positional effects.

Statistical analyses

We performed ANOVAs on our measured fitness traits when our data conformed with the assumptions that underlie parametric tests. Because our experimental design was based on specific a priori biological hypotheses, we used contrast analysis to determine significant differences in means between treatments instead of post hoc testing (Rosenthal and Rosnow, 1985). Proportional data were arcsine transformed prior to analysis. We used Kruskall-Wallis tests (corrected for ties) where our data violated the criteria for use of parametric tests.

To determine differences in female mortality rates between experimental treatments and controls we used Cox regression. This method enabled us to include both categorical and continuous covariates, and test explicit a priori hypotheses by use of appropriate contrasts.

All tests used all available data except for those involving egg-to-adult survival and total fitness. Of the 90 replicates chosen for fitness estimates, 12 females (13%) laid no eggs and a further 18 (20%) had offspring egg-to-adult survival rates of zero for unknown reasons. These females were not significantly associated with any particular treatment (G-test=0.11, df=2, P=0.95), and were removed to enable us to normalise the data for the appropriate parametric contrast analyses. The general patterns of our dataset were unaffected by the presence or removal of these individuals.

Results

Male size and mating duration

Male size did not differ between any of the three mating treatments (ANOVA: $F_{2,\ 177}$=2.33, P=0.10, using the size of the first male for those females mated to four males) nor between the four males in the polyandrous treatment (ANOVA: $F_{3,\ 236}$=0.20, P=0.90). The durations of first matings did not significantly differ between the three treatments (ANOVA: $F_{2,\ 177}$=1.00, P=0.37). A repeated measures ANOVA showed no overall significant difference in mating duration between the two multiply mating treatments ($F_{1,\ 118}$=0.67, P=0.44). There was, however, a significant difference in duration between the four matings ($F_{3,\ 354}$=4.21, P=0.006). A significant linear contrast showed an overall gradual increase in mating duration from the first to the fourth mating ($F_{1,\ 118}$=6.80, P=0.01; Fig. 1). The interaction between mating treatment and mating duration was not significant ($F_{3,\ 234}$=1.81, P=0.14).

Male or female size did not significantly correlate with mating duration in any treatment (data not shown).

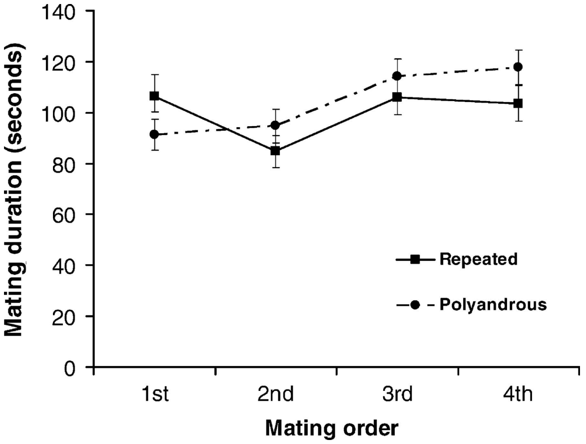

Fig. 1 Mean mating duration (±SE) for each of the four matings in both the repeated and polyandrous treatments

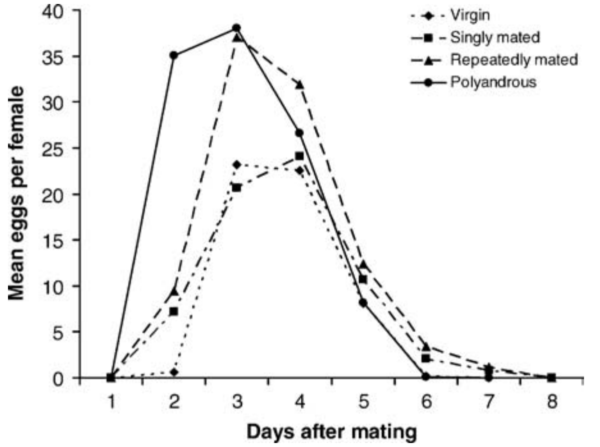

Fig. 2 Mean eggs per female for each of the three mating treatments and controls on each day after treatment. Error bars are omitted for clarity

Total lifetime fecundity and oviposition patterns over time

There was a significant linear contrast between mating treatment and the number of eggs laid (virgin<single<repeated<polyandrous; $F_{1,\ 236}=53.60$, $P<0.001$; Table 1). A quadratic contrast was not significant ($F_{1,\ 236}=0.22$, $P=0.64$). Mated females laid more eggs than virgins ($t_{236}=5.04$, $P<0.001$). Multiply mated females laid more eggs than singly mated females ($t_{236}=5.22$, $P<0.001$). Polyandrous females laid more eggs than monandrous females ($t_{236}=4.13$, $P<0.001$; Table 1). There was a significant treatment effect on the number of clutches laid (Kruskall-Wallis test: $\chi^2{}_3=25.32$, $P<0.001$), with multiply mated females tending to lay more clutches than virgin or singly mated females (Table 1).

All females laid their eggs between the second and seventh day after copulation (Fig. 2). Polyandrous females laid more eggs on the second day after copulating compared to females from the other two treatments and control females. Polyandrous females also laid relatively high numbers of eggs for 3 days, which exceeded the oviposition rates of other females. Repeatedly mated and polyandrous females laid more eggs on the third day after mating than singly mated or virgin females. After 3 days post treatment there were no differences between groups in the numbers of eggs laid on each day (Fig. 2; Table 2). The highest daily oviposition rates of singly mated and virgin females were on the fourth day after treatment. Females from the repeatedly mated and polyandrous groups, however, reduced their oviposition rates on the fourth day after mating compared to the third day (Fig. 2).

Fertility, egg-to-adult survival and lifetime reproductive success

Fertility and egg-to-adult survival rates were low across all treatments (Table 1). We found no evidence of a fertility benefit to multiple mating ($t_{177}=0.45$, $P=0.65$), but the egg-to-adult survival rates of polyandrous females' offspring were significantly higher than those of monandrous females ($t_{56}=2.58$, $P=0.013$; Table 1).

There was a significant linear contrast between mating treatment and the total number of offspring females produced (single<repeated<polyandrous; $F_{1,56}=26.80$, $P<0.001$). A quadratic contrast was not significant ($F_{1,56}=0.01$, $P=0.94$). Females that mated multiply produced more offspring than singly mated females ($t_{56}=4.44$, $P<0.001$). Females that mated polyandrously produced more offspring than monandrous females ($t_{56}=4.55$, $P<0.001$; Table 2).

Table 1 Mean (±SE) fecundity (*eggs*), number of clutches laid (*clutches*), fertility rates (*fertility*), egg-to-adult survival (*survival*), total number of offspring produced (*fitness*) and life span (*longevity*) of females from the three experimental treatments and virgin controls

	Virgin	Single mating	Repeated mating	Polyandrous
Eggs	54.60 (4.23)	63.93 (5.87)	94.85 (6.23)	109.77 (7.27)
Clutches	1.60 (0.11)	1.80 (0.14)	2.15 (0.14)	2.37 (0.14)
Fertility	–	28.25 (2.58)	29.25 (2.50)	28.83 (2.44)
Survival	–	25.97 (2.75)	26.50 (2.07)	33.69 (2.14)
Fitness	–	19.44 (3.40)	30.35 (2.63)	40.76 (2.69)
Longevity	8.00 (0.22)	6.13 (0.12)	7.63 (0.17)	6.47 (0.16)

Table 2 Kruskall-Wallis test results using the numbers of eggs laid per female between the three experimental treatments and controls on each of the 6 days of oviposition (see Fig. 2). A repeated measures ANOVA was not computed as the data for each day violated the assumptions for parametric statistical tests. P-values shown to be significant remain so after correction for multiple tests (Rice 1989)

Day	χ^2_3	*P*
Day 2	68.12	<0.001
Day 3	16.78	0.001
Day 4	4.47	0.22
Day 5	3.57	0.31
Day 6	7.12	0.07
Day 7	3.76	0.29

Table 3 Pearson's (Spearman's rank for clutch) correlation coefficients among fitness and components of fitness: the number of clutches produced (*clutch*), total number of eggs laid (*eggs*), fertility, female life span (*longevity*), egg-to-adult survival (*survival*), female size (*size*), total mating duration (*mating*), and the total number of offspring produced (*fitness*)

	Clutch	Eggs	Fertility	Longevity	Survival	Size	Mating
Fitness	0.24*	0.76**	0.03	−0.06	0.71**	0.07	0.42**
Clutch		0.55**	0.04	0.08	−0.21	−0.10	0.29*
Eggs			0.00	0.09	0.17	0.01	0.51**
Fertility				0.08	0.07	−0.12	−0.23
Longevity					−0.13	−0.01	0.17
Survival						−0.12	−0.23
Size							0.17

* $P<0.05$, ** $P<0.01$

Female longevity

We used a Cox regression to measure the effects of the three mating treatments relative to virgin controls, with female size and the number of eggs laid as additional covariates, on female mortality rates. Females that mated singly (β±SE=1.22±0.21, Wald=35.72, $P<0.001$) and polyandrous females (β±SE=0.97±0.21, Wald=20.73, $P=0.001$) had significantly higher mortality rates than virgin controls. The mortality rates of repeatedly mated females were higher than virgin controls but this was not significant (β±SE =0.34±0.20, Wald=2.90, $P=0.09$). Although the absolute difference in female longevity between the four treatment groups was small (Table 1), females that mated singly or polyandrously incurred a reduction in life span of 23% and 19% respectively, compared to virgin females. Female size (β±SE=0.04±0.12, Wald =0.12, $P=0.73$) and the total number of eggs laid (β±SE= 0.00±0.00, Wald =0.19, $P=0.66$) had no effect on female mortality independent of mating treatment.

Correlations between fitness and fitness components

The number of clutches laid, egg-to-adult survival and the total number of eggs laid were the only fitness components we measured that significantly correlated with the total fitness of females. The total time an individual female spent mating (single mating duration for singly mated females or the sum of the four matings for both groups of multiply mated females), was also significantly correlated with the total fitness of females (Table 3).A multiple regression using all four significant correlates to predict fitness showed that clutch number (β±SE=−1.56±0.90, $t_{54}=-1.73$, $P=0.09$) and total mating duration (β±SE=0.00±0.00, $t_{54}=-0.62$, $P=0.54$) did not affect fitness when the effects of egg-to-adult survival and egg number were removed. Egg-to-adult survival (β±SE=14.67±0.88, $t_{55}=16.58$, $P<0.001$) and the total number of eggs laid (β±SE=0.27±0.02, $t_{55}=14.93$, $P<0.001$) still significantly predicted overall fitness independently of each other. The significant correlations between clutch number and overall fitness, and total mating duration and overall fitness were therefore spurious, and resulted from the correlation between clutch number and the total number of eggs laid, and the correlation between total mating duration and the total number of eggs laid.

Discussion

Female *C. frigida* that mated four times had greater reproductive success than singly mated females. Mating repeatedly with the same male and mating polyandrously translated to a 56% and 110% fitness advantage compared to singly mated females, respectively. This suggests that there are both direct and indirect benefits of multiple mating to females, and both benefits are of equal importance. We found no evidence of any fertility benefits of multiple mating. As expected, however, the offspring of polyandrous females had increased egg-to-adult survival rates, and this was important in determining the overall reproductive success of females. Our data indicate that a direct benefit of multiple mating is due to the mechanism(s) that increases oviposition. Polyandrous females gain additional indirect benefits of a further elevated rate of oviposition and increased egg-to-adult offspring survival rates.

Multiple mating commonly increases fecundity in insects. This is usually attributed to increased resources transferred by males to females (Vahed 1998) but this is unlikely to occur in coelopids (Dunn et al. 2001). In many species of Diptera mating directly affects female reproductive behaviour (reviewed by Chapman et al. 1998). The physical stimuli of copulation may induce females to actively elevate their oviposition rate as a function of mating rate. Alternatively, males may manipulate female behaviour in their favour, which may conflict with female interests (Holland and Rice 1998). This effect is best known in *Drosophila melanogaster*, in which male seminal proteins induce oviposition, reduce female receptivity to future males, and elevate female mortality rates as a side-effect of these processes (Chapman et al. 1995; Wolfner 1997). Our data show that multiple mating induces females to lay eggs quickly, and to lay more eggs. The total mating time of females also increased egg production rates. This provides support for a male influence on female behaviour, and if this effect is chemical it is likely to be dose dependent. Although previous experiments have not shown any effects of mating on oviposition in *C. frigida* (Dunn et al. 2002; D.M. Shuker, cited in Chapman et al., 1998), past work has largely focussed on examining the effects of a single mating. Moreover, our data suggests that the effect of mating on oviposition is exacerbated when a female has been exposed to the ejaculates of more than one male (Fig. 2). Similar synergistic effects have been found to operate in other insects, e.g. the bean weevil *Callosobruchus maculatus* (Eady et al. 2000).

Traits in males' ejaculates that affect female reproductive behaviour are the likely result of selection driven by sperm competition (Stockley 1997; Simmons 2001). Little is known about post-copulatory events in *C. frigida* other than short-term last-male sperm precedence in twice-mated females (Day and Gilburn 1997; Blyth and Gilburn, unpublished data). Wild female *C. frigida* mate with many males (>1,000 in their lifetime; Blyth and Gilburn, unpublished data). If last-male sperm precedence persists at high rates of polyandry (but see Zeh and Zeh 1994), males may strategically allocate small amounts of ejaculate per insemination to minimise sperm/seminal fluid depletion (Pitafi et al. 1994; Pitafi 1997). Males encounter vast numbers of females around oviposition sites (Day and Gilburn 1997). The chance of being any female's last male prior to oviposition will therefore increase with the number of females inseminated, and males will benefit by inducing females to oviposit.

Our data provides no evidence that *C. frigida* males detect, or react to, the mating status of females, because there were no differences in the time males spent mating with non-virgin novel females or females they had previously inseminated. The overall gradual increase in mating duration across the four matings of both multiply mated treatments, may simply have been because it took longer for males to deposit a given amount of ejaculate due to sperm/seminal fluid depletion.

If males titrate small numbers of sperm per ejaculation, then this may explain why we found generally low fertility rates. Previous studies of *C. frigida* from different populations have found higher fertility rates than those shown in our experiment. Pitafi et al. (1994) measured egg fertility rates laid by singly mated females from an inbred laboratory strain as 60%. Crocker and Day (1987) found egg fertility rates of nearly 100% but also found a high proportion of infertile females (>50%). Butlin et al. (1984) found egg fertility rates in excess of 90%. Crocker and Day's and Butlin et al.'s experiments, however, did not prevent females from mating at very high rates. Direct quantification of sperm numbers per ejaculate, and further studies of fertilisation rates, sperm competition, and sperm utilisation patterns in different populations, are essential to understand the factors that determine such wide variation in fertilisation rates in this species.

Egg-to-adult survival was clearly important in determining the overall fitness of females but was generally low. Butlin and Day (1984) found larval survival rates between 18% and 34%, which concur with the overall mean rate (29%) in our study. We found higher egg-to-adult survivorship in the offspring of polyandrous females compared to monandrous females. In addition to the synergistic effects on oviposition, this additional benefit is likely to have contributed to the overall fitness advantage of polyandrous females. Future experiments exploring polyandry in *C. frigida* using isokaryotypic lines are likely to be useful, because egg-to-adult survival benefits associated with the αβ inversion are only predicted to be available to homokaryotypic females (see Gilburn and Day 1996).

There appears to be a general longevity cost of mating to females, independent of egg production, that may be offset by copulating repeatedly with the same male. If males transfer small amounts of nutrients to females, repeatedly mating with the same male may counteract the cost of copulation. However, the probability of wild females mating multiple times with the same male is likely to be low. An increase in female mortality rate as a result of mating is likely to be a side-effect of male adaptations that manipulate female behaviour, rather than functioning specifically to harm females (see Johnstone and Keller 2000; Morrow et al. 2003). Our data show that polyandry may have a similar synergistic effect on female longevity as it does oviposition. It is tempting to speculate that these effects are not independent but more sophisticated experiments are clearly required to clarify the process(es) involved. Moreover, a longevity cost to females exhibiting very high rates of polyandry may be much higher than that measured in our experiment if any synergistic effect is increased.

The correlates we identified as being associated with total female fitness, highlight the relative significance of female longevity as a currency to measure direct costs of mating to female *C. frigida*. Longevity did not influence the total fitness of females, presumably because oviposition is concentrated in the first few days after mating, and the cost we detected although significant was small. If any detrimental effects of mating to females only become apparent after oviposition, it is unlikely that selection will favour those females genetically able to overcome them. We suggest that future work concentrate on the factors influencing oviposition and egg-to-adult survival, with longevity reduction as a possible side-effect, as these predictors contributed to such a high proportion of the total variation in female fitness.

An important conclusion from Arnqvist and Nilsson's (2000) meta-analysis of polyandry in insects is the presence of optimal mating rates for females. Although some level of multiple mating is likely to benefit females, mating at very high rates, for example due to male coercion (Clutton-Brock and Parker 1995) or seduction (Holland and Rice 1998), may be costly. Because *C. frigida* females mate at such high rates in the wild (J. Blyth and A.S. Gilburn, unpublished data), our experiment may not have enabled females to attain anywhere near their potential total fitness. This may explain the relatively low numbers of offspring produced by all females in our experiment, when they clearly had high reproductive potential. A costly rate of mating to *C. frigida* females in the currency of total offspring produced may therefore be extremely high. Identifying an optimal mating rate for female *C. frigida*, and how this may vary between experimental populations in which costs and benefits of polyandry will differ (see Zeh and Zeh 2003), is a fruitful avenue of future investigation and is currently underway.

Acknowledgements This experiment complied with the laws of the United Kingdom. We wish to thank P. Eady and M. Knight for constructively criticising an earlier version of the manuscript, S. Abolins for help with fly culture and collecting seaweed, and K. Cruickshanks for collecting seaweed. A.S. Gilburn and J. Blyth kindly provided access to unpublished data and loaned equipment. The editor and three anonymous referees provided constructive comments that enabled us to improve the manuscript.

References

Arnqvist GA, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. Anim Behav 60:145–164

Baker RH, Ashwell RIS, Richards TA, Fowler K, Chapman T, Pomiankowski A (2001) Effects of multiple mating and male eye span on female reproductive performance in the stalk-eyed fly, *Cyrtodiopsis dalmanni*. Behav Ecol 12:732–739

Bateman AJ (1948) Intra-sexual selection in *Drosophila*. Heredity 2:349–368

Birkhead TR (2000) Promiscuity: an evolutionary history of sperm competition and sexual conflict. Faber and Faber, London

Birkhead TR, Møller AP (1998) Sperm competition and sexual selection. Academic, London

Blanckenhorn WU, Mulhauser C, Morf C, Reusch T, Rueter M (2000) Female choice, female reluctance to mate, and sexual selection on body size in the dung fly *Sepsis cynipsea*. Ethology 106:577–593

Blanckenhorn WU, Hosken DJ, Martin OY, Reim C, Tueschl Y, Ward P (2002) The costs of copulating in the dung fly *Sepsis cynipsea*. Behav Ecol 13:353–358

Butlin RK, Day TH (1984) The effect of larval competition on development time and adult size in the seaweed fly, *Coelopa frigida*. Oecologia 63:122–127

Butlin RK, Collins PM, Day TH (1984) The effect of larval density on an inversion polymorphism in the seaweed fly, *Coelopa frigida*. Heredity 52:415–423

Chapman T, Liddle LF, Kalb JM, Wolfner MF, Partridge L (1995) Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. Nature 373:241–244

Chapman T, Miyatake T, Smith HK, Partridge L (1998) Interactions of mating, egg production and death rates in females of the Mediterranean fruit fly, *Ceratitis capitata*. Proc R Soc Lond B 265:1879–1894

Clutton-Brock TH, Parker GA (1995) Sexual coercion in animal societies. Anim Behav 49:1345–1365

Crean CS (1997) Variation in female mating preference in the seaweed fly, *Coelopa frigida*. PhD thesis. University of Nottingham, UK

Crean CS, Gilburn AS (1998) Sexual selection as a side effect of sexual conflict in the seaweed fly, *Coelopa ursina* (Diptera: Coelopidae). Anim Behav 56:1405–1410

Crean CS, Dunn DW, Day TH, Gilburn AS (2000) Female mate choice for large males in several species of seaweed flies (Diptera: Coelopidae). Anim Behav 59:121–126

Crocker G, Day TH (1987) An advantage to mate choice in the seaweed fly, *Coelopa frigida*. Behav Ecol Sociobiol 20:295–301

Crudgington HS, Siva-Jothy MT (2000) Genital damage, kicking and early death. Nature 407:855–856

Cullen SJ, Young AM, Day TH (1987) The dietary requirements of seaweed flies (*Coelopa frigida*). Estuar Coast Shelf Sci 56:1311–1322

Daly M (1978) The costs of mating. Am Nat 112:771–774

Day TH, Gilburn AS (1997) Sexual selection in seaweed flies. Adv Stud Behav 26:1–57

Day TH, Dobson T, Hillier PC, Parkin DT, Clarke B (1982) Nonrandom association of enzyme and chromosomal polymorphism in the seaweed fly, *Coelopa frigida*. Heredity 44:321–326

Day TH, Foster SP, Englehard G (1990) Mating behaviour in seaweed flies (*Coelopa frigida*). J Insect Behav 3:105–120

Dunn DW, Crean CS, Wilson CI, Gilburn (1999) Male choice, male willingness to mate and body size in seaweed flies (Diptera: Coelopidae). Anim Behav 57:847–853

Dunn DW, Crean CS, Gilburn AS (2001) Male mating preference for female survivorship in the seaweed fly *Gluma musgravei* (Diptera: Coelopidae). Proc R Soc Lond B 268:1255–1258

Dunn DW, Crean CS, Gilburn AS (2002) The effects of exposure to seaweed on willingness to mate, oviposition, and longevity in seaweed flies. Ecol Entomol 27:554–564

Eady P, Wilson N, Jackson, M. (2000) Copulating with multiple mates enhances female fecundity but not egg-to-adult survival in the bruchid beetle *Callosobruchus maculatus*. Evolution 54:2161–2165

Eberhard WG (1996) Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton, N.J.

Fedorka KM, Mousseau, T (2002) Material and genetic benefits of female multiple mating and polyandry. Anim Behav 64:361–367

Gilburn AS, Day TH (1996) The evolution of female choice when the preference and the preferred trait are linked to the same inversion system. Heredity 76:19–27

Gilburn, AS, Foster SP, Day TH (1992) Female mating preferences for large male size in *Coelopa frigida*. Heredity 69:209–216

Holland B, Rice WR (1998) Chase-away sexual selection: antagonistic seduction versus resistance. Evolution 52:1–7

Hosken DJ, Blanckenhorn WU (1999) Female multiple mating, inbreeding avoidance, and fitness: it is not only the magnitude of costs and benefits that counts. Behav Ecol 10:462–464

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. Biol Rev 75:21–64

Johnstone RA, Keller, L (2000) How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. Am Nat 156:368–377

Jones TM (2001) A potential cost of monandry in the sandfly, *Lutzomyia longipalpis*. J Insect Behav 14:385–399

Keller L, Reeve HK (1995) Why do females mate with multiple males? The sexually selected sperm hypothesis. Adv Stud Behav 24:291–315

Lorch PD, Chao L (2003) Selection for multiple mating in females due to mates that reduce female fitness. Behav Ecol 14:679–686

Madsen T, Shine R., Loman J, Hakansson T (1992) Why do female adders copulate so frequently? Nature 355:440–441

Martin OY, Hosken DJ (2002) Asymmetry and fitness in female yellow dung flies. Biol J Linn Soc 76:557–563

Morrow EH, Arnqvist G, Pitnick S (2003) Adaptation versus pleiotropy: why do males harm their mates? Behav Ecol 14:802–806

Newcomer SD, Zeh JA, Zeh DW (1999) Genetic benefits enhance the reproductive success of polyandrous females. Proc Natl Acad Sci USA 96:10236–10241

Olsson M, Shine R (1997) Advantages of multiple mating to females: a test of the infertility hypothesis using lizards. Evolution 51:1684–1688

Pitafi, KD (1997) Sperm titration by male seaweed flies. Pak J Zool 27:233–240

Pitafi KD, Hewitt JJ, Gilbert FS, Day TH (1994) Male reproductive incompetence, fertility, and the cost of mating in male seaweed flies. Gen Life Sci Adv 13:9–18

Rice WR (1989) Analysing tables of statistical tests. Evolution 43:223–225

Rosenthal R, Rosnow RL (1985) Contrast analysis: focussed comparisons in the analysis of variance. Cambridge University Press, Cambridge

Rowe L, Arnqvist GA, Sih A, Krupa JJ (1994) Sexual conflict and the evolutionary ecology of mating patterns: water striders as model systems. Trends Ecol Evol 9:289–293

Shuker DM (1998) Sexual selection and sexual conflict in the seaweed fly *Coelopa frigida*. PhD thesis. University of Nottingham, UK

Shuker DM, Day TH (2001) The repeatability of a sexual conflict over mating. Anim Behav 61:755–762

Shuker DM, Day TH (2002) Mate sampling and the sexual conflict over mating in seaweed flies. Behav Ecol 13:83–86

Simmons LW (2001) Sperm competition and its evolutionary consequences in the insects. Princeton University Press, Princeton, N.J.

Stockley P (1997) Sexual conflict resulting from adaptations to sperm competition. Trends Ecol Evol 12:154–159

Stockley P. Searle JB, MacDonald DW, Jones CS (1993) Female multiple mating behaviour in the common shrew as a strategy to avoid inbreeding. Proc R Soc Lond B 254:173–179

Thornhill R, Alcock J (1983) The evolution of insect mating systems. Harvard University Press, Cambridge, Mass.

Tregenza T, Wedell N (1998) Benefits of multiple mates in the cricket *Gryllus bimaculatus*. Evolution 52:1726–1730

Tregenza T, Wedell N (2002) Polyandrous females avoid costs of inbreeding. Nature 415:71–73

Vahed K (1998) The function of nuptial feeding in insects: a review of empirical studies. Biol Rev 73:43–78

Wiklund C, Karlsson B, Leimar O (2001) Sexual conflict and cooperation in butterfly reproduction: a comparative study of polyandry and female fitness. Proc R Soc B 268:1661–1667

Wilcockson R, Crean CS, Day TH (1995) Heritability of a sexually selected character expressed in both sexes. Nature 374: 158–159

Williams GC (1975) Sex and evolution. Princeton University Press, Princeton, NJ

Wolfner MF (1997) Tokens of love: functions and regulation of *Drosophila* male accessory gland products. Insect Biochem Mol Biol 27:179–192

Zeh JA, Zeh DW (1994) Last male sperm precedence breaks down when females mate with three males. Proc R Soc B 257:287–292

Zeh JA, Zeh DW (2003) Towards a new sexual selection paradigm: polyandry, conflict and incompatibility. Ethology 109: 929–950