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Post-copulatory sexual selection and female fitness in *Scathophaga stercoraria*

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Whether sexual selection increases or decreases female fitness is determined by the occurrence and relative importance of sexual-conflict processes and the ability of females to choose high-quality males. Experimentally enforced polyandry and monogamy have previously been shown to cause rapid evolution in the yellow dung fly *Scathophaga stercoraria*. Flies from polyandrous lines invested more in reproductive tissue, and this investment influenced paternity in sperm competition, but came at a cost to immune function. While some fitness consequences of enforced polyandry or monogamy have been examined when flies mate multiply, the consequences for female fitness when singly copulated remain unexplored. Under a good-genes scenario females from polyandrous lines should be of higher general quality and should outperform females from monogamous lines even with a single copulation. Under sexual conflict, costly adaptations will afford no advantages when females are allowed to mate only once. We investigate the lifetime reproductive success and longevity of females evolving under enforced monogamy or polyandry when mating once with males from these selection regimes. Females from polyandrous lines were found to have lower fitness than their monogamous counterparts when mating once. They died earlier and produced significantly fewer eggs and offspring. These results suggest that sexual conflict probably drove evolution under enforced polyandry as female fitness did not increase overall as expected with purely good-genes effects.

Keywords: sperm competition; polyandry; monogamy; reproductive success; longevity; *Scathophaga*

1. INTRODUCTION

Classically, sexual selection has been argued to increase fitness (Darwin 1871), but this need not necessarily be the case (Holland 2002). While there is evidence that sexual selection increases some fitness components (Petrie 1994; Welch *et al.* 1998), other studies find the opposite is true (Holland & Rice 1999; Holland 2002; Moore *et al.* 2003). Therefore, the question of whether sexual selection increases or decreases female fitness remains largely unanswered and may depend on the relative importance of sexual conflict and classical sexual selection.

Classical sexual-selection theory suggests that females base mate choice on direct fitness benefits, general male quality (i.e. good genes) or perhaps 'sexy' males (i.e. sexy-son benefits) (Andersson 1994). Under sexual conflict however, sexually antagonistic evolution (Rice & Holland 1997) means that females are enticed into mating suboptimally with males bearing extreme traits (Holland & Rice 1998). It has, however, been argued that the direct cost to females of mate choice and part of the sexually antagonistic evolution driven by sexual conflict are likely to be compensated for by sexy-son benefits (e.g. Andres & Morrow 2003; Cordero & Eberhard 2003; Pizzari & Snook 2003). Unfortunately for these arguments, sexy-son benefits may not influence evolutionary equilibria (Kirkpatrick 1985, 1988; Gavrilets *et al.* 2001; Cameron *et al.* 2003; Pizzari & Snook 2003), and therefore suggestions that benefits via sons can compensate females for direct fitness costs may be incorrect. It has also been suggested that good-genes and sexy-son benefits form a continuum depending on how mating success trades off against

survival (Kokko *et al.* 2002). However, this idea has since been questioned (Cameron *et al.* 2003). Importantly, the small indirect benefits females typically gain from sexual selection (e.g. Møller & Alatalo 1999) will frequently not offset larger direct costs (Kirkpatrick & Barton 1997), so the detection of large direct fitness costs to females as a result of sexual selection appears to be reasonably sound evidence for conflict-driven evolution. Nevertheless, sexual conflict and classical sexual-selection processes can be difficult to disentangle (Parker 1979; Cordero & Eberhard 2003; Andres & Morrow 2003; Pizzari & Snook 2003), but both can be strengthened through polyandry (Andersson 1994; Birkhead & Møller 1998; Holland & Rice 1998).

Polyandry is the norm in insects, and there are a number of direct and indirect benefits females may gain from mating with more than one male (Keller & Reeve 1995; Arnqvist & Nilsson 2000; Jennions & Petrie 2000; Hosken & Stockley 2003; Radwan 2003). Nevertheless, multiple mating may also impose a number of costs on females (e.g. Crudgington & Siva-Jothy 2000; Blanckenhorn *et al.* 2002*a*; reviewed in Stockley 1997). Polyandry (in contrast to strict monogamy) will also lead to increased sexual conflict and can generate evolutionary arms races between the sexes, where adaptations in males reduce female fitness and lead to the evolution of female counteradaptations (Parker 1979; Rice 1996; Holland & Rice 1998; Parker & Partridge 1998). Experiments with *Drosophila melanogaster* support this and have shown that enforced monogamy leads to the evolution of more benevolent males and that when females are prevented from coevolving with males, male fitness increases at a cost to females (Rice 1996; Holland & Rice 1999; but see also Pitnick & Garcia-Gonzales 2002). Similar results were found in the fly *Sepsis cynipsea* (Martin & Hosken

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2003*b*). Increased investment in reproductive processes may lead to trade-offs, energy allocated to reproduction having to be diverted from other functions (Roff 2002). For instance, offspring quality could increase owing to sexual selection, but have to be traded off against offspring number (Madsen & Shine 1998). Indeed, there is evidence for trade-offs between traits involved in sperm competition and life-history traits (Civetta & Clark 2000; Hosken 2001).

Post-copulatory sexual selection has been studied extensively in the yellow dung fly *Scathophaga stercoraria* (L.) (Parker 1970; Simmons & Parker 1992; Ward 1993, 2000; Simmons *et al.* 1999; Hosken *et al.* 2002; Tregenza *et al.* 2003). Recent evidence has shown that experimentally enforced polyandry and monogamy have rapid evolutionary consequences in this species (Hosken & Ward 2001; Hosken *et al.* 2001; and see Snook 2001). Polyandrous flies invest more in reproduction (increased size of testes and female reproductive accessory glands), which increases their ability to influence paternity during sperm competition (Hosken *et al.* 2001). However, there is an immune-system cost to this (Hosken 2001). Male fitness consequences of enforced evolution have been examined (polyandrous males are better sperm competitors), but the consequences for female fitness remain to be firmly established. It is clear that flies in enforced polyandrous lines underwent evolutionary changes resulting from the opportunity for post-copulatory sexual selection and sexual conflict. Indeed, these changes seem to afford both sexes more control over paternity in sperm competition. If these changes are driven by female choice for high-quality males, we would expect polyandrous flies generally to be of higher genetic quality (NB: cryptic female choice has been documented in yellow dung flies; Ward 2000). If, however, changes are sexually antagonistic adaptations driven by sexual conflict, they will not necessarily increase overall quality. Polyandrous flies should then outperform monogamous counterparts under their own (i.e. competitive) conditions but not when post-copulatory sexual selection is absent.

The purpose of this study was twofold. First, the study examines the general consequences of enforced polyandry for female fitness. Second, and more specifically, we ask whether the evolutionary changes documented in these selection lines are more likely to be driven by female choice for high-quality males or by sexual conflict over paternity. These questions were addressed experimentally by maintaining replicate monogamous and polyandrous lines of *S. stercoraria* (both treatments with random mate assignment and no opportunity for pre-copulatory sexual selection). After 10 generations, females were allowed to mate once with males from these treatments, and female lifetime reproductive success (fecundity, fertility), longevity and offspring quality (larval competitive ability) were quantified. Importantly, as the focus here is on disentangling good-genes from conflict processes, females were allowed to mate only once. This experimental treatment is necessary to measure the costs of female adaptations to enforced polyandry. If females were allowed to mate multiply, i.e. under conditions that polyandrous females are adapted to, costs could be obscured by any potential benefits afforded by greater control over paternity. If adaptations to post-copulatory sexual selection are driven

by good-genes processes, we expect polyandrous females generally to be of better quality and to have greater fitness than monogamous females, even when females mate singly. Under a conflict scenario, polyandrous females should not be of higher general quality and should outperform their monogamous counterparts under polyandrous, but not monogamous, conditions.

2. MATERIAL AND METHODS

(a) *Copulation and female fitness*

The parental generation for the experimental flies was founded using eggs of female *S. stercoraria* collected at Fehraltorf near Zurich. These were divided into two treatments: monogamous (M) and polyandrous (P) lines (four replicate lines of each treatment; for details see Hosken & Ward 2001). Flies copulated once in M lines and three times in P lines, and flies were killed after females had laid eggs. As flies were killed soon after copulating, this regime did not select on female longevity, since a female's lifespan did not influence the number of her offspring in the next generation. Therefore, in terms of longevity we do not expect selection for more harmful males or more resistant females in the polyandrous lines. These lines were maintained for 10 generations before conducting the experiments described here. Experimental flies were housed for three weeks prior to copulations. To examine the fitness consequences of experimentally enforced polyandry and monogamy, females from each treatment were allowed to copulate once with males from these selection regimes. A further experiment comparing monogamous females with females originating from crosses between monogamous lines was carried out at generation 12 (females mated with P or M males as above) to control for potential inbreeding differences between P and M lines. For all copulations, males and females were placed in vials with a portion of dung (standard procedure; for details see Ward & Simmons 1991). Copulation duration was measured to the nearest minute, and males were removed (and frozen at $-80\,°C$ after recording hind tibia length (HTL) as a measure of body size) as soon as copulations had ended. Note that the size of females did not differ between treatments (using cross type as the replicate and HTL as the dependent variable: $F_{1,14} = 4.16$; $p = 0.61$; mean HTL for monogamous-treatment females: 2.55 ± 0.08 mm; for polyandrous-treatment females: 2.72 ± 0.04 mm).

After copulation, females were left in the vials to lay a clutch in the dung. Eggs were counted under a stereomicroscope; 10 eggs were taken from each clutch and placed on small pieces of filter paper on 20 g of dung in a closed container. The number of eggs that hatched was assessed 48 h later. Containers were then housed under controlled optimal conditions and the number of emerging offspring were subsequently counted. After laying, females were housed singly in vials with sugar, water and *Drosophila* and checked daily for the duration of the experiment (i.e. until all females had died). All flies and dung containers were kept in a climate chamber at $15\,°C$ and 60% relative humidity. Every second week females were given a small portion of dung and allowed to lay overnight. Again, eggs were counted and 10 eggs were placed in containers with 20 g of dung for counts of hatchability and emergence. Dead females were frozen and longevity (days) was recorded. HTL was measured for each female.

(b) *Larval competitive ability*

We used larval competitive ability as an estimate of offspring quality. Offspring of females from each treatment competed against each other. For this, an additional 10 eggs were collected from each female's first clutch. These were placed on 15 g of dung (restricted enough to allow competition; Amano 1983) in closed containers with 10 eggs from a female of the other treatment, both females having copulated with males from the same treatment (i.e. male × female: P × P versus P × M and M × P versus M × M; 10 replicates of each cross-type were performed). Flies were left to emerge and were frozen at −80 °C. DNA from these flies and from the parents (to establish paternal and maternal genotypes) was extracted using a QIAmp DNA Mini Kit tissue protocol. Offspring genotypes were assigned to potential parents on the basis of polymorphic DNA microsatellites (Garner *et al.* 2000) using an Elchrom SEA 2000 electrophoresis system with Spreadex gels.

(c) *Statistical analysis*

Data were analysed using general linear models (GLM) for fecundity, fertility and longevity data. Separate analyses were conducted using either flies or line means as the unit of replication, as the latter analysis has few degrees of freedom and therefore low power. The outcomes of the larval-competition assays were analysed with a Wilcoxon signed-rank test, and were analysed as inbred versus outbred flies regardless of line of origin, although we note here that lines were represented in a reasonably balanced way. The residuals of dependent variables in the GLM analysis were checked and were normally distributed (Kolmogorov–Smirnov test).

3. RESULTS

(a) *Copulation and female fitness*

Multivariate GLM analysis with egg number, number of eggs hatching, number of offspring emerging and longevity as dependent variables was performed with male and female type as factors and male and female HTL as covariates. Analysis indicated that female treatment had a significant multivariate effect (female type: Wilks' lambda $F_{4,17} = 3.60$, $p = 0.027$). All other multivariate effects were non-significant (Wilks' lambda $F_{4,17} < 1.89$, $p > 0.159$). Univariate analysis indicated that the multivariate effect of female type was driven by its significant effect on all four dependent variables (effect on egg number: $F_{1,20} = 6.02$, $p = 0.024$; on number of hatched eggs: $F_{1,20} = 14.52$, $p = 0.001$; on offspring number: $F_{1,20} = 14.39$, $p = 0.001$; on longevity: $F_{1,20} = 6.37$, $p = 0.020$; table 1). Females from the polyandrous treatment laid fewer eggs, produced fewer offspring and died sooner than females from monogamous lines (table 1). Male HTL was weakly positively associated with number of hatched eggs ($\beta = 1.76$, $F_{1,20} = 4.65$, $p = 0.043$). None of the other predictors or male × female interactions had a significant effect on any of the dependent variables (all $F_{1,20} < 2.63$, all $p > 0.12$). Importantly, the differences between female treatments concern lifetime reproductive success (i.e. total egg and offspring numbers). Although females from monogamous lines did not lay more eggs in the first two clutches, they outdid females from polyandrous lines by surviving longer and laying more eggs overall. Similarly, mean hatch and emergence rates do not differ between treatments but total numbers of hatched

Table 1. Mean ± s.e. longevity, fecundity and fertility by cross type.

(Abbreviations (male × female): MM, monogamous × monogamous; MP, monogamous × polyandrous; PM, polyandrous × monogamous; PP, polyandrous × polyandrous. The numbers of hatched eggs and emerging offspring are low compared to egg numbers because they were obtained from subsets of 10 eggs for each clutch laid. Discrepancies between numbers of hatched eggs and emerging offspring are the result of hatching eggs being assessed 48 h after they were laid. Eggs that took longer to hatch were therefore not included in these counts but will, nevertheless, be apparent in the final number of offspring to emerge.)

longevity after copulation (days)	
MM ($n = 15$)	30.67 ± 5.69
MP ($n = 16$)	27.88 ± 5.44
PM ($n = 16$)	38.25 ± 3.64
PP ($n = 16$)	32.00 ± 4.05
total eggs (counting only females that laid)	
MM ($n = 7$)	106.43 ± 12.85
MP ($n = 8$)	82.88 ± 8.88
PM ($n = 7$)	112.71 ± 14.04
PP ($n = 7$)	92.00 ± 15.75
lifetime number of hatched eggs	
MM ($n = 7$)	22.00 ± 3.20
MP ($n = 8$)	12.50 ± 1.75
PM ($n = 6$)	20.17 ± 2.04
PP ($n = 6$)	13.17 ± 2.50
mean proportion to hatch	
MM ($n = 7$)	0.81 ± 0.04
MP ($n = 8$)	0.83 ± 0.04
PM ($n = 6$)	0.82 ± 0.07
PP ($n = 6$)	0.85 ± 0.04
lifetime number of live offspring	
MM ($n = 7$)	18.76 ± 2.51
MP ($n = 8$)	10.25 ± 1.62
PM ($n = 6$)	17.33 ± 1.61
PP ($n = 6$)	10.83 ± 2.27
mean proportion to emerge	
MM ($n = 7$)	0.83 ± 0.04
MP ($n = 8$)	0.81 ± 0.07
PM ($n = 6$)	0.87 ± 0.04
PP ($n = 6$)	0.82 ± 0.08

eggs and emerging offspring are greater in females from the monogamous lines (table 1).

Using cross-type means (means for each male–female line combination) instead of data for individual females did not qualitatively change the results. Analysis again indicated a significant multivariate effect of female treatment (female type: Wilks' lambda $F_{4,7} = 4.54$, $p = 0.04$). All other multivariate effects were non-significant (Wilks' lambda $F_{4,7} < 3.03$, $p > 0.096$). Univariate analysis indicated that the multivariate effect of female type was driven by its significant effects on three out of the four dependent variables, with females from the polyandrous lines producing fewer eggs and offspring (table 1) (effect on egg number: $F_{1,10} = 6.18$, $p = 0.03$; on number of hatched eggs: $F_{1,10} = 18.97$, $p = 0.001$; on offspring number: $F_{1,10} = 12.26$, $p = 0.006$). However, the effect on longevity was no longer significant, although the trend was in the same direction as before with females from the polyandrous treatment tending to die sooner ($F_{1,10} = 4.04$,

$p = 0.07$). Male HTL was positively associated with number of hatched eggs ($\beta = 2.80$, $F_{1,10} = 7.22$, $p = 0.02$), and number of hatched eggs was negatively associated with female HTL ($\beta = -2.86$, $F_{1,10} = 5.88$, $p = 0.036$).

It is important to note that egg number is typically positively associated with body size in insects (Andersson 1994), including the yellow dung fly (Parker 1970), and that females from polyandrous lines were slightly (though not significantly) larger than their monogamous counterparts (see § 2a). In spite of this, however, these larger females (from the polyandrous lines) laid fewer eggs. Additionally, in free-living flies, longevity is positively associated with body size (Hosken *et al.* 2003), but again we found that larger females (from the polyandrous treatment) had reduced longevity in one of our analyses.

In the above analyses, sample sizes for MANCOVAs were greatly reduced as only females that laid eggs were entered into the models. Therefore, separate ANCOVAs were performed with longevity as the dependent variable and including all females (whether they laid or not). Male and female type and egg laying (yes or no) were entered as factors, and male and female size were entered as covariates. The interaction between egg laying and female type was significant with egg-laying polyandrous females dying earlier than egg-laying monogamous females ($F_{1,48} = 4.78$, $p = 0.03$; all other effects n.s.: $F_{1,48} < 2.25$, $p > 0.14$). Using size difference (male HTL − female HTL, because effects could be caused by the relative size of males and females) as a covariate instead of male and female body sizes did not alter these results ($F_{1,49} = 5.85$, $p = 0.02$) and indicated a trend for a size-difference effect ($F_{1,49} = 3.62$, $p = 0.06$, $\beta = 0.91$; all other effects n.s.: $F_{1,49} < 2.46$, $p > 0.12$). This indicates that polyandrous females die earlier than monogamous females only if they reproduce. Again using cross-type means did not affect the results, with the interaction still being significant when size difference was used (female type × egg laying: $F_{1,23} = 4.63$, $p = 0.04$) but not when male and female HTL were used ($F_{1,22} = 3.06$, $p = 0.09$).

We used the same GLM approach to analyse the comparison of monogamous and monogamous-crossed females to see whether inbreeding was likely to be a problem. There was no significant effect of female type (or any other predictor), either in multivariate (Wilks' lambda $F_{4,20} < 1.10$, $p > 0.382$) or in univariate ($F_{1,23} < 1.74$, $p > 0.20$) analyses. In any event, in the main experiment described above, the monogamous females, which would be expected to be more inbred, had significantly greater fitness. Consequently, there is no reason to believe that inbreeding effects caused the patterns detected.

Thus, we found that reproductive success and longevity (in one analysis) of females from the polyandrous treatment are decreased compared with females from the monogamous lines when females mate once. Longevity determined the number of eggs laid, which in turn determined the number of offspring produced. Effects were independent of mate type with no indication that polyandrous males were more harmful to their mates (table 1). However, the lack of a male effect is consistent with expectations, as the experimental selection regime did not select on longevity or male malevolence.

(b) *Larval competitive ability*

A Wilcoxon signed-rank test failed to show a significant effect of female type on larval competitive ability ($n = 16$, T+ = 90.50, T− = 45.50, $Z = 1.16$, $p = 0.122$). The reduction in sample size (16 instead of the initial 20) is a result of the lack of emerging flies in three competition trials and to equal numbers of offspring from both mothers emerging in one trial. These results indicate that differences in egg and offspring numbers reported above are not caused by differences in larval competitive ability.

4. DISCUSSION

Females that evolved under polyandry were found to have lower fitness than their monogamous counterparts when mated once. Decreases were found in all measures of reproductive success scrutinized: females from polyandrous lines died earlier (or showed a strong tendency to die earlier, depending on the analysis) and produced significantly fewer eggs and offspring. The decrease in longevity was critical, as it determined the number of clutches a female laid, in turn affecting offspring numbers. The reduction in the reproductive success of females from polyandrous lines is consistent with findings in *Drosophila*. Holland & Rice (1999) found that monogamous populations had a greater net reproductive rate than control populations with sexual selection. However, they also cautioned that this need not always be the case, as the costs of sexual conflict do not necessarily exceed the potential benefits of sexual selection. Our results suggest that here the costs do exceed the benefits when females evolving under enforced polyandry do not reproduce under conditions of sperm competition. The decreased fitness in the polyandrous lines may be explained in terms of the previously documented rapid evolutionary changes. While the sizes of the spermathecae of females from the two selection regimes did not differ, females from polyandrous lines evolved larger accessory glands (Hosken *et al.* 2001). Additionally, flies evolving under polyandry had reduced immune function (Hosken 2001). Either or both of these effects could explain the costs seen. However, as this was not explicitly tested, a direct causal link has not been established. Nevertheless, the differences in lifetime reproductive success are stark.

The apparent reduction in longevity was detected in females from polyandrous lines that laid eggs; when females did not reproduce, there was no effect. Therefore, reduction in immune function *per se* is an insufficient explanation for the decreased longevity, as one would then expect to see non-reproducing females from these lines also die earlier. However, an impoverished immune system may prove costly as soon as females reproduce, because mating and oviposition can lead to periods of immune suppression, rendering females more susceptible to infection (e.g. Siva-Jothy *et al.* 1998; Rolff & Siva-Jothy 2002).

Both male and female adaptations in the polyandrous lines are specific to a sperm-competition context, and, in a non-competitive situation, females paid a cost for their microevolutionary changes in investment. As no general increase in female quality was found, it appears that the evolutionary changes previously documented were not the

result of cryptic female choice for higher-quality males. Cryptic female choice can influence paternity in free-living yellow dung flies, although it apparently does not result in directional selection (Ward 2000). Instead, it appears that the changes in the lines are conflict-driven adaptations aimed at gaining the upper hand in male–female conflict over paternity. Our results also show that males evolving under polyandry are not of higher quality under monogamous conditions, since their offspring did not have increased performance. Again, sexual conflict over paternity could explain this result. Fisherian processes could also be involved (Parker 1979), and it is conceivable that polyandrous females benefit indirectly via increased sperm competitiveness of their sons (Keller & Reeve 1995), especially given the experimental evolution protocol. Furthermore, and more generally, it has been proposed that, when benefits via sons are factored into female fitness, the costs of sexual conflict and sexually antagonistic coevolution are negated and net fitness benefits may accrue to females that mate with overly attractive males (e.g. Cordero & Eberhard 2003; Eberhard & Cordero 2003). However, this is still a matter of much debate (e.g. Pizzari & Snook 2003) and, specifically, it is unclear what, if any, effect indirect benefits via sexy sons can have on the evolutionary outcome of sexual selection (Kirkpatrick 1985, 1988; Gavrilets *et al.* 2001; Cameron *et al.* 2003; Chapman *et al.* 2003). Population genetics models suggest that evolutionary equilibria remain unchanged when benefits via sons are included (Kirkpatrick 1985, 1988; Gavrilets *et al.* 2001), and furthermore, mutational-bias sexy-son models may in fact portray good-genes processes (Cameron *et al.* 2003). If sexy-son effects are negligible, the increased ability of females from polyandrous lines to influence paternity that was documented previously (Hosken *et al.* 2001) must be attributed to selection for increased variance within clutches. Nevertheless, if any sexy-son effect exists, females from our polyandrous treatments could profit from it. However, we note that benefits would have to be extremely large to outweigh the drastic reduction in female lifetime reproductive success, which seems extremely unlikely (Kirkpatrick & Barton 1997; Møller & Alatalo 1999; Cameron *et al.* 2003).

The lack of a male effect on egg or offspring production might seem intriguing in view of the greater investment in male reproductive tissue in polyandrous lines (enlarged testes) and the ability of these males to achieve higher paternity (Hosken *et al.* 2001; Hosken & Ward 2001). However, it is important to bear in mind that in the current experiment females were allowed to mate only once. Therefore, effects of male adaptations to sperm competition will not be apparent. Additionally, males from polyandrous lines may be selected to ejaculate prudently despite larger testis size (e.g. Gage 1991; Gage & Baker 1991; Martin & Hosken 2002a; Wedell *et al.* 2002). In any case, the sizes of female sperm-storage organs (spermathecae) did not differ between treatments (Hosken *et al.* 2001), indicating that females evolving under polyandry are not able to store more sperm. The experimental protocol used for the monogamous and polyandrous selection also did not select on longevity, as flies were killed as soon as females had laid one clutch of eggs. Accordingly, there was no selection on male benevolence or malevolence (*sensu* Rice 1996). Therefore, the lack of a male effect on longevity was expected, and does not contrast with previous findings in *Drosophila* (Rice 1996; Holland & Rice 1999). Similarly, as there was no selection on female longevity, we do not expect polyandrous females to be more resistant to mating harm. Male size had a positive (but weak) effect on the number of hatched eggs. A negative effect of male body size on egg number had previously been documented in this species (Martin & Hosken 2002b), but was not supported in a larger study (Blanckenhorn *et al.* 2002b). Because of this disparity and the weakness of the effect found here, the influence of male size on female reproduction is equivocal and requires further investigation.

The evidence presented here is consistent with previous work on these selection lines. The morphological changes documented previously, which were interpreted as being caused by increased sperm competition and sexual conflict (Hosken & Ward 2001; Hosken *et al.* 2001), are not advantageous when females copulate only once. In a non-competitive situation, polyandrous females apparently fail to profit from their adaptations. Although a causal link was not established, the decrease in female fitness seems to be a direct consequence of these and possibly of immune-system changes (Hosken 2001). Polyandrous females did not outperform monogamous females, as might be expected if cryptic female choice for high-quality males or good-sperm effects had driven these rapid evolutionary changes. We suggest that these changes have probably been driven by sexual conflict. However, recent work has found that superior sperm competitors sire faster-developing young (Hosken *et al.* 2003), but again the selection conditions here would not have allowed this benefit to be manifest. Taken together these studies support the notions that experimental perturbation is often required for the detection of sexually antagonistic evolution and that conflict and more classical sexual-selection driven evolution can occur simultaneously (Holland & Rice 1998, 1999; Rice 2000; Arnqvist & Rowe 2002; Nilsson *et al.* 2002; Martin & Hosken 2003a,b).

In summary, we find not only that the opportunity for sexual selection and conflict drives rapid evolutionary changes in reproductive traits, as shown previously, but also that these changes affect female fitness components. Females evolving under experimentally enforced polyandry have lower reproductive success and decreased longevity compared with females evolving under monogamy when both types of female mate once. Adaptations to increased polyandry impose irreversible costs on females and seem to be profitable only in a competitive situation.

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