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MATERNAL EFFECTS ON OFFSPRING DEPEND ON FEMALE MATING PATTERN AND OFFSPRING ENVIRONMENT IN YELLOW DUNG FLIES

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Abstract.—Direct costs and benefits to females of multiple mating have been shown to have large effects on female fecundity and longevity in several species. However, with the exception of studies examining genetic benefits of polyandry, little attention has been paid to the possible effects on offspring of multiple mating by females. We propose that nongenetic effects of maternal matings on offspring fitness are best viewed in the same context as other maternal phenotype effects on offspring that are well known even in species lacking parental care. Hence, matings can exert effects on offspring in the same way as other maternal environment variables, and are likely to interact with such effects. We have conducted a study using yellow dung flies (*Scathophaga stercoraria*), in which we independently manipulated female mating rate, number of mates and maternal thermal environment and measured subsequent fecundity, hatching success, and offspring life-history traits. To distinguish between direct effects of matings and potential genetic benefits of polyandry we split broods and reared offspring at three different temperature regimes. This allowed us to demonstrate that although we could not detect any simple benefits or costs to matings, there are effects of maternal environment on offspring and these effects interact with female mating regime affecting offspring fitness. Such interactions between female phenotype and the costs and benefits of matings have potentially broad implications for understanding female behavior.

Key words.—Cryptic female choice, maternal effects, multiple mating, polyandry, *Scathophaga*, *Scatophaga*, sexual conflict.

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Understanding the causes and consequences of female mating frequency remains a major challenge for evolutionary biology. The consequences of multiple matings to females have been divided into two classes: genetic effects on offspring fitness (Jennions and Petrie 2000) and direct effects on female fecundity and lifespan (Arnqvist and Nilsson 2000). A possibility that has not been investigated is that female multiple mating may affect offspring indirectly in the same way that other aspects of maternal environment can affect offspring fitness (Mousseau and Fox 1998). For example, in many insects, including yellow dung flies, maternal diet influences egg size and hence offspring quality (Fox 1993; Rossiter et al. 1993; Jann and Ward 1999), and maternal effects have also been shown to affect offspring development time and size at adulthood (reviewed in Roff 1992). There is evidence that such effects are likely because it is known that substances acquired by females in matings can affect offspring fitness (Vahed 1998). However, it has yet to be shown directly that female mating frequency can affect offspring fitness in the absence of genetic benefits, and the potential for interactions between maternal effects due to matings and other aspects of maternal environment has not been investigated.

Matings inevitably have costs for females, taking time and energy, and exposing the female to diseases and potentially harmful effects of attempted manipulation by males. Therefore, to understand female mating behavior, we need to understand the associated costs and benefits. This applies even to species, in which females apparently have little control over whether to mate, because this is likely to reflect a situation where there is no great benefit to females of avoiding matings. As well as incurring costs, females may gain direct benefits from matings, enhancing their own reproductive success, and potentially the fitness of their offspring, through processes such as egg provisioning or paternal care. Alternatively, benefits of matings may be genetic, with offspring of polyandrous females receiving better or more compatible genes. Genetic benefits have received a quite a lot of attention (see Jennions and Petrie 2000; Hosken and Stockley 2003, for reviews). However, although the existence of direct costs and benefits is universally acknowledged, less effort has gone into measuring these effects and to placing them in the context of other environmental factors affecting female fecundity and offspring fitness. The best studied examples of direct costs and benefits of matings are cases when mating rate affects female lifespan and fecundity either through costs of matings due to physical or chemical injury or through benefits due to acquisition of nutrients from males (Arnqvist and Nilsson 2000). We have carried out a study designed to allow us to distinguish between direct and genetic benefits of matings and to examine interactions between direct effects of matings on offspring and the influence of other environmental effects affecting females. Our aim is to place non-genetic effects of matings on offspring fitness in the context of other effects of maternal environment.

The yellow dung fly, *Scathophaga* (sometimes *Scatophaga*) *stercoraria*, is a naturally polyandrous species. Males gather on fresh dung pats and seize females as they arrive to lay eggs (Parker 1970a), hence females typically alternately mate and oviposit. Copulation occurs prior to oviposition with the last male to mate fathering around 80% of the subsequent clutch (e.g., Parker 1970b; Ward 2000). Nevertheless, most females ($> 90\%$) arriving to oviposit are nonvirgins, and the sperm stored from a single copulation ensures full fertility for about four clutches (Parker et al. 1993). Although males have a high degree of control over matings, females have multiple spermathecae (Ward 1993), mediate sperm storage (Simmons et al. 1999; Hosken and Ward 2000), and are able to differentially store (Otronen et al. 1997) and use sperm (Ward 2000). There is evidence that female *S. stercoraria* can exercise postcopulatory choice according to male genotype and environmental temperature (Ward 1998; Ward 2000). Larval performance and pupal mass vary depending on genotype by temperature interactions (Ward 1998), suggesting that cryptic female choice may enable females to increase offspring fitness by using sperm from males whose genotype is suited to prevailing environmental conditions (Ward et al. 2002). Strong maternal effects on offspring fitness have also been documented (Jann and Ward 1999).

To examine effects of mating regime, including maternally mediated effects due to costs and benefits of matings *per se* in the yellow dung fly, we conducted a study in which females were maintained at three different temperature regimes and: (1) mated once to one male, (2) twice to one male, or (3) once each to two males, and their offspring divided and reared at three different temperature regimes. This design allows us to examine effects of number of matings by comparing treatments 1 and 2, and the effects of mating to two different males by comparing treatments 2 and 3. Maintaining females under different environmental conditions also allows us to examine maternal effects that arise from environmental effects on adult females.

Our study was designed to test several specific hypotheses:

1) *Are there nongenetic benefits or costs of matings?* These include effects on females such as avoidance of sperm limitation and acquisition of nutrients or oviposition stimulants in the ejaculate, and effects on offspring through maternal effects. These would predict differences in egg laying, fertility, or offspring traits between females mated once and females mated twice.

2) *Are there genetic benefits of polyandry for embryonic viability?* If polyandrous females have more viable offspring, as found in similar studies in other animals (see Jennions and Petrie 2000; Tregenza and Wedell 2000; Zeh and Zeh 2001), females mated to two different males should have higher offspring viability than those mated twice to the same male.

3) *Do offspring of polyandrous females have less variation in fitness related traits across thermal environments?* There is genetic variation in optimal larval development temperature in yellow dung flies (Ward 1998). By splitting broods between different temperature regimes and examining whether polyandrous females have offspring with less variation in fitness related traits across environments, we can test the hypothesis that polyandry is beneficial because it increases offspring diversity.

4) *Are there maternal effects on offspring life histories?* The effects on offspring of maternal environment demonstrate the potential for offspring fitness to be affected by costs and benefits to the female of mating. Maternal size, nutrition, and age all influence egg size, offspring survival, and size at adulthood (Jann and Ward 1999), but more subtle effects such as the influence of adult female environment have not been investigated.

5) *Are there interactions between maternal effects and mating regime affecting offspring viability and life histories?* Interactions between maternal effects and mating regimes may occur because females in different conditions may experience different levels of costs or benefits from matings. For instance, males might invest less in females reared under suboptimal conditions, or such females might experience greater costs of attempted manipulation by males. Effects of this type would predict interactions between female mating regime and female rearing temperature affecting offspring viability and life histories.

MATERIALS AND METHODS

Female dung flies had eclosed at least 14 days prior to our experiments. All females were virgins derived from a stock reared in the laboratory for five generations using standard methods (Ward 1993): 16 h light period, 8 h dark photoperiod, and a larval temperature of 16°C. Following emergence, females were kept at one of three temperature regimes: (1) constant 11°C; (2) constant 19°C; or (3) a temperature set at 22°C during the light period and 13°C during the dark period (the ''variable'' regime). These assorted temperature regimes create differences in maternal condition with potential implications for maternal effects. For example, it has been shown that egg volume is negatively related to temperature from 11–23°C in both the laboratory and field, and smaller eggs typically perform poorly (e.g., have higher mortality). Additionally, egg size increases in the wild as temperatures fall from September to November (Blanckenhorn 2000), and it is also probable that maternal rearing temperature influences egg quality. As with all ectotherms, larval development time is also positively associated with temperature, but performance is maximized at intermediate temperatures. Laboratory studies have shown that in *S. stercoraria*, a rearing temperature of 19°C is associated with low mortality and fast development time (Blanckenhorn 2000). The 11°C constant temperature treatment approximates spring and fall temperatures at low altitude sites in Switzerland where mean temperature is about 8–11°C and mean variation is very small, whereas the higher variable temperature simulates summer conditions (high mean and greater variation) (Blanckenhorn 1997).

Male flies were caught on and around dung pats near Fehraltorf, Switzerland (the same population from which females were originally sourced) two days prior to being used in the experiments, and were then kept in individual vials provided with excess food (sugar crystals and live *Drosophila*) and water. Thirty-six females from each maintenance temperature were allocated at random to one of three treatments: (1) one mating to one male; (2) two matings to the same male; (3) one mating to each of two different males.

To control conditions for mating, egg laying, and rearing as much as possible, on day one, 36 females were allocated to each of groups 2 and 3 and were each mated once. On day two, females from groups 2 and 3 were mated a second time, and a further 36 females allocated to group 1 were also mated. The 24 h interval between matings creates an opportunity for potential direct costs or benefits of mating to influence the females' eggs in the doubly mated groups. On both days, matings of females from the different treatment groups were temporally alternated to control for possible effects of time of mating. Matings were staged by placing a male and female together in a vial in a sunlit window. The vial contained a

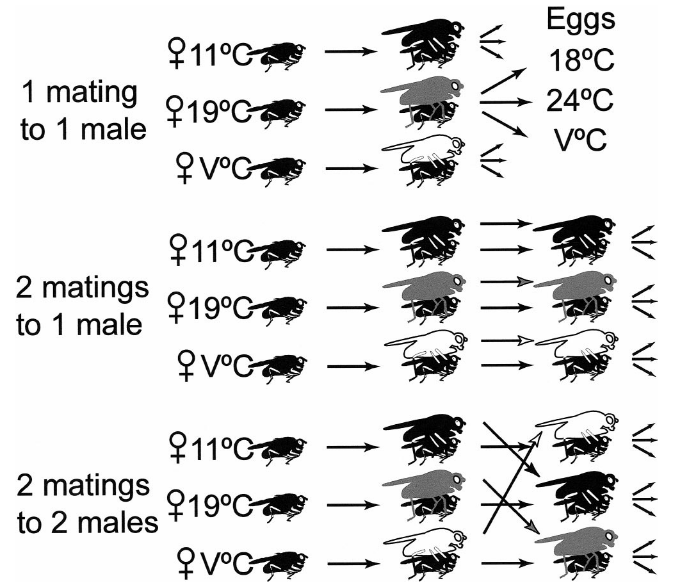

FIG. 1. Experimental design. There are three mating treatments, all of which use females maintained under three different temperature regimes. In treatment 1 females mate only once; in treatment 2 they mate twice to the same male; in treatment 3 they mate twice to two males, and males are switched between females so there are no systematic differences in male mating experience across treatments. Flies in treatment 1 are allocated their single mating on the same day as flies in treatments 2 and 3 mate for the second time, so eggs are laid at the same time across treatments. Three batches of 10 eggs from each female are then allowed to hatch and the offspring reared to adulthood at each of three temperature regimes.

piece of filter paper with a smear of cow dung that helps stimulate copulation, presumably because matings naturally occur in close proximity to dung. To control for differences in the mating of males, each treatment group used the same number of males as females, so males in group 3 were switched between females while those in group 2 mated again to the same female (Fig. 1). Immediately following their last mating on day two, females were placed in a vial with a teaspoonful of dung and allowed to oviposit at 21°C. Females typically lay all their mature eggs in a single clutch (Jann and Ward 1999). Once they had ceased oviposition, females were placed in an Eppendorf tube and frozen at −20°C. Any females that failed to oviposit were placed in a vial with food and water and left overnight before being given additional laying opportunities one and five days later. Eleven females died without laying eggs and two failed to lay any eggs. All these females were excluded from subsequent analyses.

Immediately after oviposition, we counted the number of eggs produced (under a microscope). We divided 30 eggs from each female equally between three damp pieces of filter paper and discarded the remainder. The pieces of filter paper and eggs were placed into containers with overabundant dung for the developing larvae (> 2 g/larva) (Amano 1983). Vials were divided between incubators set at 16 h light, 8 h dark, and at three different temperature regimes: (1) constant 18°C; (2) constant 24°C; (3) and a variable regime in which the 16 h light period was at 20°C and the 8 h dark period was at 14°C. These temperature regimes differ from those used to rear the parental females because we wanted to ensure maximum survivorship of mothers and also maximize the probability of detecting effects in the offspring. At 11°C pre-emergent flies typically enter diapause until the temperature increases, so although adults can be maintained at 11°C, directly developing offspring cannot be reared at this temperature. Similarly, adult flies could not be maintained at 24°C or they would have died too quickly. The variable temperature regime was intended to produce a mean development time similar to 18°C despite a lower mean temperature (Blanckenhorn 1999), because development time decreases faster than linearly with temperature, although in fact flies developed slightly faster under the variable than the 18°C regime (Table 1).

After 48 hours we checked eggs under a microscope and counted the number that had hatched successfully by recording empty chorions. The majority of eggs hatch after around 24 h (Parker 1970a), so our approach gives an unbiased estimate of hatching success, which is likely to be a slight underestimate because a small proportion may hatch subsequently when it is difficult to observe them because of fungal growth on the dung surface. Emerging larvae crawled off the filter paper and burrowed into the dung. Subsequently, we monitored vials daily, removing newly eclosed adults, recording their sex, mass, and development time (egg laying to emergence).

Because our male parental generation flies were wild, their mass will depend on recent nutrition; hence, rather than mass, we used hind-tibia length as a measure of size in parents: This correlates well with overall body size (Ward and Simmons 1991), and was measured using a dissecting microscope and graticule.

RESULTS

Are There Direct or Indirect Effects of Mating Regime? (Hypotheses 1 and 2)

Egg laying and hatching success.—The number of eggs laid did not differ between treatment groups (mating regime and

TABLE 1. Offspring life-history traits (mean with standard errors in parentheses). Mating treatments are pooled. Numbers of adults emerging are from a potential maximum of five individuals of each sex. N = the number of sibling groups from which subsequent means are taken. See text for details of rearing regimes.

Offspring rearing temperature regime	N	Number of adult males	Number of adult females	Male mass/mg	Female mass/mg	Male development time/days	Female development time/days
18°C	63	4.25 (0.19)	3.90 (0.16)	25.33 (0.40)	15.72 (0.24)	27.24 (0.12)	26.00 (0.13)
24°C	48	4.02 (0.21)	3.65 (0.17)	18.37 (0.41)	11.78 (0.21)	20.32 (0.13)	19.34 (0.11)
Variable	55	3.95 (0.22)	4.49 (0.19)	21.80 (0.36)	13.46 (0.21)	28.79 (0.18)	27.52 (0.19)

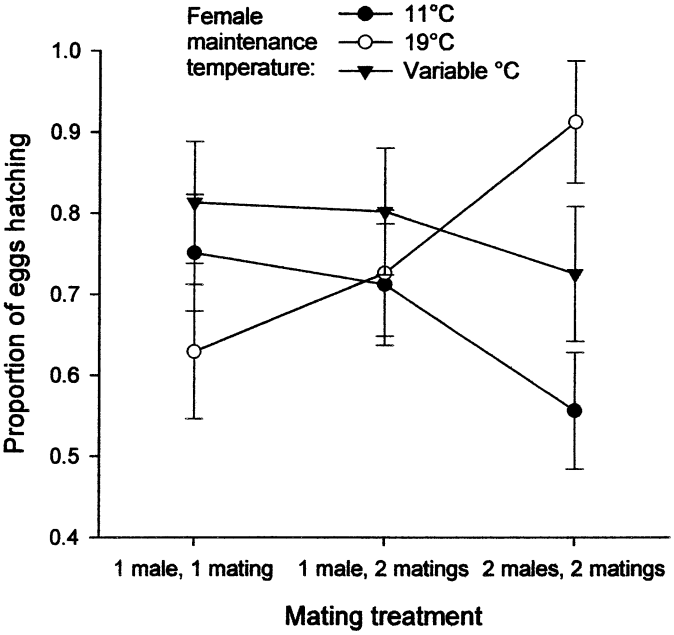

FIG. 2. The relationship between mating treatment, maternal environment, and egg hatching success. Bars are standard errors.

maternal temperature regime) and the interaction was also nonsignificant, (two-way analysis of covariance (ANCOVA) with female size as a covariate: mating regime $F_{2,85} = 1.40$, $P = 0.25$; female environment $F_{2,85} = 0.68$, $P = 0.94$; interaction $F_{4,85} = 0.80$, $P = 0.53$; there was a strong effect of female size $F_{1,91} = 12.3$, $P = 0.001$; mean number of eggs ± SE = 55.5 ± 1.76). Similarly the proportion of eggs hatching did not differ between treatment groups (main effects in ANOVA with mating regime and female environment as factors and reflected square root transformed, square root (constant –x), mean hatching success: mating regime $F_{2,86} = 0.39$, $P = 0.68$; female environment $F_{2,86} = 1.94$, $P = 0.15$). There was a significant interaction between female environment and mating regime (Fig. 2), described below.

Offspring life history traits.—Of our original 36 females in each mating treatment, 21, 22, and 20 females in groups 1, 2, and 3 respectively had offspring of both sexes emerging as adults from all three offspring temperature regimes. Only data from these individuals were used in our analyses, as there is no satisfactory way of dealing with missing data points in repeated measures analyses. For each female, we have three broods reared at different temperatures, within which we have three life-history measures: (1) the number of individuals of each sex emerging as adults (from the 10 eggs); (2) their mean mass; and (3) development time. We did not adjust emergence by the number of eggs observed to have hatched because of the possibility that our measure of hatching success is an underestimate (see Methods); therefore, our successful emergences measure includes hatching success, although the previous analyses indicate that hatching success does not vary between treatments. There were no significant outliers in the data and results of evaluation of assumptions of normality, homogeneity of variance-covariance matrices, linearity, and multicollinearity were satisfactory. We conducted a doubly multivariate repeated measures multivariate analysis of variance (MANOVA) using SPSS, with mating treatment (3 groups) and female environment (3 groups) as factors and number of adults emerging, development time, and adult mass as dependent variables (measures) with the three offspring rearing temperatures as the first level repeated factor, and sex of offspring as a factor within temperature. Maternal size was included as a covariate, as size is known to be heritable in this species (Simmons and Ward 1991). There was indeed a strong effect of maternal size on offspring life histories, the multivariate combination of our three measures, ($F_{3,51} = 6.26$, $P = 0.001$). However, there were no differences between mating regimes in offspring life histories ($F_{6,102} = 1.32$, $P = 0.26$). This is not a Type-II error resulting from incorporating both maternal maintenance temperature and mating regime in a single experimental design. If a one-way repeated measures MANOVA is conducted with group as the only factor, there is still no effect ($F_{6,114} = 1.72$, $P = 0.12$).

Do Offspring of Polyandrous Females Have Less Variation in Fitness-Related Traits across Thermal Environments? (Hypothesis 3)

Because each female's offspring were split and reared at three different temperatures, we can test this hypothesis by comparing the variance in offspring life-history traits across temperatures between mating treatments (ignoring female maintenance temperature). Mean values of life-history traits were standardized such that within each temperature the mean for each trait was 0 and the standard deviation was 1.0. We then calculated the standard deviation (SD) of offspring trait values across the three temperature regimes for each female. We used these SD's in a repeated measures MANOVA (offspring sex being the repeat) using all three life-history traits (survival, mass, and development time) as the dependent variables and mating treatment as the factor. This revealed an effect of mating treatment on variability of life-history traits across temperatures ($F_{6,152} = 2.73$, $P = 0.015$). A planned contrast indicated that this effect is driven by differences between offspring of polyandrous and those of monandrous females ($F_{3,76} = 4.70$, $P = 0.005$). Amongst traits, offspring survival differed most between mating treatments ($F_{1,78} = 7.69$, $P = 0.007$), with less effect of offspring development time ($F_{1,78} = 3.66$, $P = 0.059$), and no significant variation in offspring mass ($F_{1,78} = 0.83$, $P = 0.37$). However, opposite to our prediction, polyandrous females show more variation in offspring life histories across temperatures than monandrous females. Offspring survival had a SD across temperatures of 0.78 in polyandrous females and 0.59 in monandrous females. For development time, polyandrous mean SD was 0.74 while monandrous was 0.61.

Maternal Effects on Life Histories (Hypothesis 4)

Egg laying and hatching success.—There were no significant main effects of female environment on egg laying (see Hypotheses 1 and 2) or on egg hatching success, although there were interactions with mating regime (see Hypothesis 5).

Offspring life history traits.—Although the MANOVA of mating treatment and female environment versus offspring

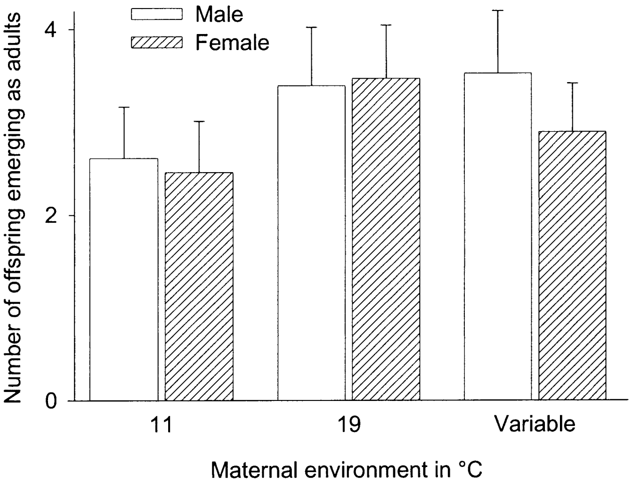

FIG. 3. The relationship between maternal maintenance temperature and number of offspring emerging as adults. Maternal environment influences offspring survival controlling for maternal size through an interaction between offspring sex and maternal rearing temperature. Bars are standard errors.

life-history traits (above) did not reveal any differences between mating regimes, it did show a strong effect of maternal environment on combined offspring life-history traits ($F_{6,104} = 3.16$, $P = 0.007$). Univariate between-subjects tests on the three life-history variables revealed that female environment affected the number of offspring that emerge as adults ($F_{4,54} = 6.38$, $P = 0.003$) (Fig. 3), but not their mass ($F_{4,54} = 0.29$, $P = 0.89$) or development time ($F_{4,54} = 1.56$, $P = 0.20$). This effect is unlikely to be due to differences in hatching success because this did not vary with female environment (see above).

Because the effect of maternal environment could be a result of differences in body size between females maintained at different temperatures, we added female size as a covariate to the analysis. Female size did have a significant effect on offspring life-history traits ($F_{3,51} = 6.26$, $P = 0.001$), but controlling for this did not change the significance of the maternal maintenance temperature effect ($F_{6,102} = 4.11$, $P = 0.001$). Maternal size influences all the life-history traits we measured: 18 separate regressions of maternal size against offspring mass, development time, and number emerging. These show that r^2 values are similar across traits. In all three traits, regression slopes are in the same direction (across both sexes and three temperatures): as maternal size increases, the number of adults emerging decreases, development time increases, and body mass increases.

Unsurprisingly, there were differences in offspring life histories between rearing temperature regimes (multivariate within-subjects effect $F_{6,49} = 849$, $P < 0.001$) and between sexes (multivariate within-subjects effect $F_{3,52} = 492$, $P < 0.001$); a summary of offspring life-history data is given in Table 1.

Interactions between Maternal Effects and Mating Regime (Hypothesis 5)

Egg hatching success.—There was a significant interaction between mating treatment and female maintenance temperature affecting the proportion of eggs hatching (Fig. 2) (2-way analysis of variance (ANOVA), mean square root reflected transformed proportional hatching success across the three batches of 10 eggs per female, interaction $F_{4,86} = 3.40$, $P = 0.013$; Mean proportional success ± SE = 0.74 ± 0.026). Outliers do not drive this effect, transformed data were normally distributed, and excluding females in which all eggs failed to hatch in at least one batch of 10, does not change the effect ($F_{4,76} = 3.68$, $P = 0.009$). Main effects were nonsignificant: mating regime $F_{2,86} = 0.39$, $P = 0.68$; female environment $F_{2,86} = 1.94$, $P = 0.15$.

Offspring life history traits.—Our analysis of offspring life-history traits (see Hypotheses 1 and 2) found no interaction between female environment and mating regime affecting the multivariate combination of offspring life-history traits ($F_{12,138} = 1.29$, $P = 0.23$).

DISCUSSION

This study reveals that offspring fitness is affected by complex interactions between aspects of the environment experienced by their mother—in this case, the temperature at which she was maintained and her pattern of mating. Additionally, we found that offspring rearing temperature had a greater effect on the life histories of progeny from polyandrous females than those from monandrous females. These findings illustrate the fact that mating behavior is only one factor affecting females and their offspring, and that different factors may combine to affect fitness.

Direct and Indirect Effects of Mating Regime

Our finding that there are no differences between mating treatments in the number of eggs laid is consistent with previous studies in which females were mated different numbers of times to the same male (Stockley and Simmons 1998; Hosken et al. 2002). Female dung flies clearly do not respond to mating more frequently or to mating with two males by laying larger clutches, although such behavior has been shown in several other insect species (Markow et al. 1990; Wilson et al. 1999). This may simply be because females do not visit dung pats until they have a clutch of eggs ready for oviposition and are constrained to lay all their available eggs in a single laying episode.

We found no independent effect of mating treatment on egg viability. Increases in egg and offspring viability in polyandrous females have been shown in several other species including crickets (Tregenza and Wedell 1998; Simmons 2001), reptiles (Madsen et al. 1992; Olsson et al. 1994), birds (Kempenaers et al. 1999; but see Whittingham and Dunn 2001), and mammals (Keil and Sachser 1998). No such benefits are found in this study, suggesting that polyandry in dung flies is not driven by viability benefits to offspring due to co-inheritance of alleles conferring success in sperm competition and offspring viability, or through genetic compatibility effects (Zeh and Zeh 1996). Additionally, females clearly do not suffer from a lack of sperm to fertilize their first clutch when mated to only one male, and previous work indicates full fertility is maintained over four clutches with one copulation (Parker 1970a).

We found no differences between mating regimes in the mean or variance of three offspring life-history traits: survival to adulthood, adult mass, and development time between regimes (controlling for differences between offspring rearing temperatures). Any effects on female condition caused by mating treatment are clearly not sufficiently large to be detected, as in the majority of previous studies that also show no differences between offspring of polyandrous and monandrous females once they have hatched or been born (see Jennions and Petrie 2000; Tregenza and Wedell 2000; Zeh and Zeh 2001). This is in contrast, however, to Eady et al. (2000) that found that female bruchid beetles mating to different males had reduced offspring survival relative to those mating repeatedly to the same male.

Polyandry and Variation in Offspring Fitness across Environments

When controlling for effects of offspring rearing temperature by using the three temperatures as repeated measures, we found no difference between the offspring of females experiencing different mating regimes in the overall mean or variance in life-history traits. However, when we explicitly examined the level of variation in offspring life-history traits across temperatures within a brood, we found strong effects of female-mating regime. Contrary to our prediction that offspring of polyandrous females might show less variation in performance across environments, our study found greater within family variation across rearing temperatures in offspring of polyandrous females, even though there was no overall mean difference between mating treatments. This result is unexpected and contradictory to the hypothesis that polyandry has genetic benefits to females through increasing the range of environments in which offspring can thrive. One possible explanation is there are some males with genes that confer higher than average fitness at a particular temperature, but lower than average at other temperatures, and other males with genes that have intermediate fitness across temperatures. If there are such temperature specialists and generalists, and the specialists are more likely to prevail in sperm competition (or due to cryptic female choice; Ward 1998), then polyandrous females might show more variation in offspring performance across temperatures, as observed. However, further studies will clearly be needed to test this hypothesis. It is worth noting that the fitness implications of increased variance in offspring life-history traits depend on the relationship between trait value and fitness. Increased variance is associated with increased fitness only when this relationship is concave-up (Hosken and Blanckenhorn 1999), which is unlikely for the traits we measured.

Maternal Effects on Offspring Life Histories

Maternal environment had a strong effect on the number of offspring that emerged as adults (Fig. 3), as females maintained at 11°C had lower offspring emergence than the other temperatures. A likely explanation for this pattern is there are maternal effects on larval diapause, as has been demonstrated in several other insects, including dipterans (reviewed by Mousseau and Dingle 1991). Although such an effect has not previously been observed in *Scathophaga*, an increase in diapause frequency in offspring of females maintained at lower temperatures is exactly the pattern seen in species such as the flesh fly *Sarcophaga bullata* (Denlinger 1998). Another possibility is that there may be differences in female investment of eggs at different temperatures. Previous work has shown that mothers reared under nutritional stress have lower egg hatching success and lower survival of offspring to adulthood (Jann and Ward 1999). If female maintenance temperature affects metabolic efficiency, then one might expect effects on offspring survival. Maternal size also affects offspring life-history traits. Larger mothers have larger daughters, with longer development times and lower emergence. This may be due to nongenetic maternal effects, such as those resulting from female maintenance temperature, but is perhaps more likely to reflect heritable variation in these traits. Development time and mass are known to be heritable and positively correlated in this species (Blanckenhorn 1998). The decrease in number of offspring surviving to adulthood with increased maternal size is intriguing because it represents a potential cost to large female size that may counterbalance the many benefits to large size in this species (e.g., Borgia 1981; Jann et al. 2000).

Interactions between Maternal Effects and Mating Regime

Because matings invariably have costs and benefits, these effects may interact with other influences on female fitness, notably environmental factors. Such interactions would mean that optimal mating strategies differ between females according to their phenotypic condition. We found an interaction between female environment and mating regime, affecting egg-hatching success. Fig. 2 indicates that the interaction is primarily driven by the difference between females maintained at 19°C and those maintained at 11°C or under a variable regime, and through greater influence of maternal temperature on the egg viability of polyandrous females. The cause of this difference is unclear. Perhaps only females maintained under optimal conditions are able to benefit from polyandry through increased fertilization success of genetically superior or more compatible males. Clearly, further investigations are needed, particularly because if there are indeed differences in costs of mating based on female phenotypic condition, this will create selection on females to make state dependent decisions (McFarland 1977) over matings, with broad implications for sexual selection.

Conclusions

Our study shows that the effect of female-mating regime on offspring fitness is dependent on complex interactions with other maternal effects and offspring environment. Although this makes it difficult to put any straightforward interpretation on our results, they clearly reveal that there are subtle costs and benefits of female-mating pattern that may only be seen when a species's ecology, particularly the environment of both mother and offspring, is taken into account. Past studies have concentrated on the potential for genetic benefits of polyandry and the effects of female-mating pattern on her own fecundity. This study shows we should also consider how the costs and benefits to females of matings may affect offspring fitness, and how these effects may interact with other maternal effects.

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