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Variation in polyandry and its fitness consequences among populations of the red flour beetle, *Tribolium castaneum*

Aditi Pai · Stacy Feil · Guiyun Yan

Abstract Female mating with multiple males in a single reproductive period, or polyandry, is a common phenomenon in animals. In this study we investigated variation in female mating behavior and its fitness consequences among three genetic strains of the red flour beetle, *Tribolium castaneum*. We found that the extent of polyandry and its fitness consequences varied significantly among the strains. In the first strain *PRUZ*, females mated multiply but incurred costs of polyandry in the form of reduced offspring production. Females of the second strain, *NDG*11, mated readily with multiple partners and benefited because polyandry led to higher offspring quality. Finally, *TIW*1 females were resistant to multiple mating and polyandry resulted in lower offspring production but improved offspring quality. Thus, in the first population we observed only costs of polyandry, in the second strain only benefits of polyandry whereas in the third we detected both costs and benefits of polyandry. Possible explanations for such a pattern are discussed.

Keywords *Tribolium castaneum* · Fitness · Polyandry · Male–female co-evolution

Introduction

Female mating behavior has attracted much attention from evolutionary biologists in the past two decades (e.g. Ridley 1988; Andersson 1994; Jennions and Petrie 1997,

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2000; Arnqvist and Nilsson 2000; Birkhead 2000). Progress in our understanding of female mating behaviors such as female mate choice and multiple mating has revolutionized sexual selection theories (Eberhard 1996). It is evident that sexual selection through female mate choice may be a major force in speciation (Wu et al. 1995; Gray and Cade 2000; Boughman 2001; Ting et al. 2001; Masta and Maddison 2002). Similarly, study of female mating with multiple partners (polyandry) has led to the realization that post-copulatory processes, namely sperm competition and cryptic female choice, may have profound impacts on the evolution of species (Eberhard 1996; Birkhead 2000).

Polyandry may intensify the conflict of interests between the sexes. Whereas males are selected to maximize their paternity, females may be selected to choose the best possible sire for their offspring (Birkhead 2000). Conflict of interests may lead to antagonistic co-evolution between the sexes, and recent studies show that this is a significant force leading to divergence among populations, reproductive isolation, and possibly speciation (Rice 1996; Parker and Partridge 1998; Holland and Rice 1999; Arnqvist et al. 2000; Andrés and Arnqvist 2001; Hosken et al. 2001; Gavrilets et al. 2001; Arnqvist and Rowe 2002; Chapman et al. 2003). Hence, the study of polyandry and its fitness consequences is important because it can provide insights into the nature of sexual conflict and the evolution of the species.

The storage pest *Tribolium castaneum* (red flour beetle) has been widely used as a model system for studying questions relating to polyandry (Bernasconi and Keller 2001, Pai and Yan 2002a, 2003, Pai et al. 2005) and sexual conflict (Nilsson et al. 2002, 2003; Attia and Tregenza 2004). Several studies have independently shown that populations of red flour beetles have diverged with respect to reproductive traits by conducting crosses between individuals from different populations (Nilsson et al. 2002, 2003; Pai and Yan 2002b, Attia and Tregenza 2004) and revealed that male and female genetic background influence various aspects of reproductive success such as mating rate (Nilsson et al. 2002), oviposition rate (Attia and Tregenza 2004), and sperm precedence (Pai and Yan 2002b, Nilsson et al. 2003). Such a pattern of population divergence has also been shown in other insects (Andres and Arnqvist 2001, Brown and Eady 2001, Harano and Miyatake 2005). Possible explanations for the divergence among populations include differences in genetic background and difference in environments which could result in test populations experiencing different evolutionary forces.

This study determined the effects of genetic background on female multiple mating behavior and its fitness consequences in three populations of *T. castaneum*, collected from different geographical regions. These populations have been maintained in the same laboratory for several years and are presumably well adapted to laboratory conditions. Because the behavior and fitness testing was conducted under the same environmental conditions, differences of mating behavior in these populations reflect genetic differences possibly due to different evolutionary ecological forces in the original populations.

Methods

Beetle rearing

We used four *T. castaneum* strains: *NDG*11, *TIW*1, *PRUZ*, and c*SM*. Strains *NDG*11, *TIW*1, and *PRUZ*, provided by Dr. Richard Beeman (Biological Research

Unit Grain Marketing and Production Research Center, KS), have different geographic origins and are genetically distinct (Beeman et al. 1996, Beeman and Brown 1999). Strain cSM was provided by Dr. Michael Wade, Department of Biology, Indiana University (Wade 1977). These strains have been maintained as separate cultures in our laboratory for more than 5 years at population sizes of 200 or more. Beetles were raised in 8-dram shell vials containing ~5 g standard medium (95% fine sifted whole wheat flour and 5% dried powdered brewer's yeast) and maintained in a dark incubator regulated at $29°C$ and 70% relative humidity. To ensure virginity of beetles used in the experiments, sexes were separated as pupae.

Magnitude of polyandry

We determined the magnitude of polyandry in the three strains (*TIW*1, *NDG*11, and *PRUZ*) by determining the number of males that a female mated with in 1 h (Pai and Yan 2003). The experiment was conducted in a mating arena, a 35-mm diameter plastic Petri dish lined with filter paper and a thin layer of flour under dim light. A male from the same population was introduced into a mating arena with a virgin female. Beetles were 1–2.5 weeks post-emergence. Beetles of the three populations used in the experiments were of comparable ages. As soon as the pair completed copulation and separated, the male was removed and a fresh male was introduced into the mating arena. The process was repeated for 1 h and we recorded the exact number of partners that females mated with within that time period. A total of 36–42 females were examined for each of the three strains. A small number of males were reused between trials after a minimum 72 h recovery period.

F_1 offspring production and sex ratio from monandrous and polyandrous females

Individual virgin females, 2–4 weeks post-emergence, were given the opportunity to mate with one, two, four, eight or 16 virgin males placed simultaneously in a vial with ~5 g of flour medium for 10 days. This experimental design allowed for male–male interactions and female choice, both of which may be important in determining indirect consequences of female multiple mating. The overall copulation frequency of females is expected to be similar for all treatments (Pai and Yan 2003; Hardling and Kaitala 2005), but the degree of polyandry for females with more available males is expected to be higher than for those with fewer available males. The actual number of males that a female copulated with was not recorded because the recording process would disrupt pre-copulatory female choice or male–male inter-actions. After 10 days all adults were removed from the vials and the eggs were allowed to grow to adults for ~ 7 weeks. The number of F_1 adults was counted, and the sex ratio of offspring from each female was determined. We set up four (*NDG*11) or five (*TIW*1 and *PRUZ*) replicates for each treatment. Two *TIW*1 females that did not produce any offspring were excluded from the analysis.

F_1 offspring fitness assay

Fitness was measured as the proportion of offspring contributed by a focal F_1 individual in a test population using body color as a genetic marker (Yan and Ste-vens 1995). The fitness assay involved placing the focal individual in a population

with four other adults of the same sex and five of the opposite sex (Pai and Yan 2002a). The focal individual had the wild-type red body color (genotype +/+) and the other individuals had black body color (c*SM* genotype b/b). The black body color is determined by a co-dominant allele. Thus, all the progeny of the focal individual would be heterozygous (+/b), brown, and phenotypically distinguishable. The adults were allowed to mate and lay eggs for 10 days, after which they were removed from the vials. Eight weeks later, the contents of each vial were sifted, and the number of individuals of each genotype was recorded. The focal beetle is expected to produce 1/5 (0.20) of the offspring if all beetles in a population have equal reproductive success. Thus, the relative fitness of the focal beetle was calculated, defined as the proportion of the red beetle's offspring in a test population divided by the expectation (0.20). We examined the relative fitness of 25 F_1 males and 25 F_1 females from monandrous and polyandrous mothers for each of the three strains (*TIW*1, *NDG*11, *PRUZ*). Focal beetles were 1–10 weeks post-emergence, and rival beetles were 1–7 weeks post-emergence. Beetles used in this experiment were of comparable age and mating history (all beetles were virgins).

F_1 offspring fitness component assay

To understand the mechanism leading to fitness differences in F_1 offspring from the different mating treatments (1, 2, 4, 8 or 16 available males), we examined three fitness components. These were: male ability to inseminate available females, F_1 egg and adult progeny production, and egg-to-adult survival of F_2. The experiments described below compared fitness correlates for the same five mating treatments as those used in the fitness assay.

Insemination capacity of F_1 males

To examine mating vigor in male offspring, 15 virgin F_1 males were randomly selected from each mating treatment (1, 2, 4, 8 or 16 available males) of each strain. An individual F_1 male was placed in a 35 mm diameter plastic Petri dish in 1 g flour medium with five virgin females of the same strain (for *TIW*1 and *PRUZ* males) or of the black strain (c*SM* b/b) when same–strain beetles were not available (for *NDG*11 males). Males were removed from the Petri dish after 30 min and females were transferred into individual dishes with 1 g flour. After 3 weeks, we examined the dishes for the presence/absence of larvae to determine how many of the five available females had been successfully inseminated. A successful insemination was defined as one that led to the female producing viable offspring as indicated by the presence of larvae. Focal males ranged from 4 to 7 weeks and females were ~4 weeks old. Beetles used within an experiment (each strain) were of comparable age.

Fecundity, F_2 adult production, and F_2 egg-to-adult viability

This experiment determined whether the progeny from sons and daughters of monandrous and polyandrous mothers differed in egg-to-adult viability. Egg-to-adult viability is defined as the proportion of eggs that successfully develop into adults. One F_1 virgin male or female, 6–9 weeks old, was paired with a virgin beetle of the opposite sex 1–4 weeks of age for 24 h in a 35 mm diameter plastic Petri dish

with ~1 g of double sifted flour medium. *TIW*1 and *PRUZ* individuals were paired with same-strain partners whereas *NDG*11 individuals were paired with c*SM* b/b beetles due to unavailability of same-strain beetles. Mating partners of *TIW*1 and *PRUZ* were marked with a green marker to facilitate separation of males from females (Pai and Yan 2002b) whereas mating partners of *NDG*11 beetles had a black body color that facilitated separation of males and females. Beetles used in an experiment (each strain) were of comparable age.

Males were removed from the Petri dish and females were allowed to lay eggs for 48 h. The majority of female *PRUZ* and *NDG*11 beetles did not lay eggs in the 48 h following the set-up of the experiment, so all females in these two assays were allowed to lay eggs for an additional week. After that, the number of eggs or larvae produced by each female was counted. They were then transferred to tubes with ~5 g fresh flour medium and allowed to grow for 6 weeks. The number of adult progeny was counted, and the proportion of eggs that developed to adulthood was determined. There were 15 replicates for each treatment (1, 2, 4, 8 or 16 available males) for each sex of each strain.

Data analysis

The data on the magnitude of polyandry were analyzed using the likelihood ratio test with strain as the independent variable (SAS 1995). For all other experiments, the data for each strain were analyzed separately.

Data on F_1 offspring number from polyandrous and monandrous females were first tested for normality and homogeneity of variances and then analyzed with an Analysis of Variance model (ANOVA), with mating treatment as the independent variable. The sex ratio of F_1 offspring was tested against the null hypothesis that the proportion of male and female progeny was equal, using χ^2 test.

Interactions between male and female genotypes play an important role in a male's share of paternity (Pai and Yan 2002b), and the three strains likely differ in their interaction with the c*SM* (b/b) genotype used in the fitness assays. Therefore, it was more appropriate to analyze the data from the three strains separately for the fitness assay and the fitness component assay. To compare the relative fitness of F_1 males and females from monandrous and polyandrous mothers, we used ANOVA with sex and mating treatment as independent variables. As before, assumptions of ANOVA were tested and data were transformed when necessary. If the ANOVA yielded significant results, the post-hoc Tukey–Kramer Honestly Significant Difference (HSD) test was used to determine statistical differences among the five mating treatments within each strain. We used this approach rather than a priori tests because the effects of multiple mating may be dose dependent (Arnqvist and Nilsson 2000). This makes it difficult to predict the effect of polyandry with respect to the dose (number of mates) for each strain a priori. The assays in which the focal individuals did not produce any offspring were excluded from the analysis (8% for *NDG*11, 7% for *TIW*1, and 3% for *PRUZ*).

To compare fitness correlates of F_1, multivariate analysis of variance (MANOVA) was performed with mating treatment as a factor and fecundity, adult offspring production, and egg-to-adult survival as dependent variables. Data were transformed when necessary. In addition, univariate ANOVAs for each of the fitness correlates were also conducted, with mating treatment as the fixed factor. The assays in which no eggs were produced (6 % in *NDG*11, 10 % in *TIW*1, and 17% *PRUZ*)

were excluded from the analysis. Because each strain and sex was analyzed separately, we did not standardize the fecundity data for time for female *PRUZ* and *NDG*11 beetles. As before, the Tukey–Kramer Honestly Significant Difference (HSD) test was used to determine statistical differences among the five mating treatments within each sex for each strain. All analyses were conducted using the JMP computer program (SAS 1995).

Results

Magnitude of polyandry

Females from the three populations differed significantly in their readiness to copulate. In *NDG*11 females, 17% (6 out of 36), in *PRUZ* females, 11% (4 of 36), and in *TIW*1 females, 67% (28 of 42) did not copulate (likelihood ratio test, $\chi^2_{2, 111} = 30.10$, $P < 0.001$). Females that did not mate were excluded from the remaining analyses. The strains also varied significantly in the proportion of females that mated with more than one male (likelihood ratio test, $\chi^2_{2, 73} = 47.16$, $P < 0.001$, Table 1) and the number of males that females copulated with within 1 h (ANOVA, $F_{2, 73} = 12.59$, $P < 0.0001$). *NDG*11 females mated on average with ~4 males and a maximum of 12 males, *PRUZ* females mated with an average of ~4 males and a maximum of eight males, whereas *TIW*1 females never mated with more than one male in the 1 h observation period (Table 1).

F_1 offspring production and sex ratio from singly and multiply mated females

*NDG*11 females that were exposed to single and multiple partners during the 10 days mating period did not differ in the number of F_1 adult offspring (ANOVA, $F_{4, 15} = 2.1$, $P = 0.12$; Fig. 1). However, offspring production of *TIW*1 females was adversely affected by multiple male treatments (ANOVA, $F_{4, 18} = 5.5$, $P < 0.01$; Fig. 1). Specifically, females placed with a single male produced significantly more offspring than females placed with eight partners (Tukey–Kramer HSD test, $P < 0.05$). Similarly, offspring production of *PRUZ* females was also negatively affected by access to multiple mates (ANOVA, $F_{4, 20} = 3.17$, $P = 0.03$; Fig. 1). Females placed with a single male produced significantly more offspring than females with 16 partners (Tukey–Kramer HSD test, $P < 0.05$). The sex ratio in the F_1 populations within each treatment did not differ significantly (χ^2 tests, $P > 0.05$ for all five treatments in all three strains).

F_1 offspring fitness assay

The fitness of F_1 offspring from *NDG*11 females exposed to single and multiple partners differed significantly (Table 2). Offspring from females with 16 available

Table 1 The magnitude of polyandry among three strains of the red flour beetle, *T. castaneum*

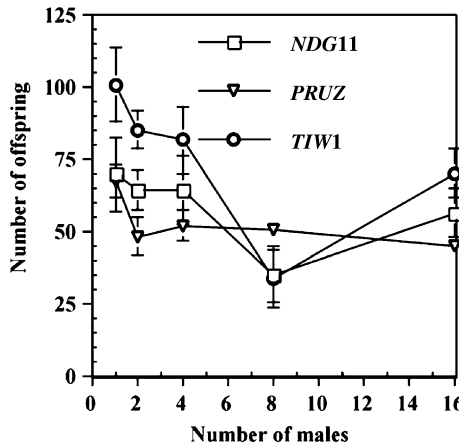| Strain	n	Mean number of copulations	Standard error	Range
*NDG*11	30	4.1	0.41	1–12
*TIW*1	14	1.0	0	1–1
PRUZ	32	3.5	0.34	1–8

Fig. 1 Offspring production by monandrous and polyandrous females in three strains of *T. castaneum* beetles (mean and standard error are shown)

partners had significantly higher fitness than offspring from females with 1, 2, or 4 available partners (Tukey–Kramer HSD test, $P < 0.05$; Fig. 2a). In *TIW*1, mothers with multiple partners also had offspring with significantly higher fitness (Table 2). Offspring from females with 16 and 4 available partners had significantly higher fitness than offspring from females with 1 or 2 available partners (Tukey–Kramer HSD test, $P < 0.05$; Fig. 2c). However, multiple partners did not significantly affect the fitness of F_1 offspring in the *PRUZ* strain (Table 2, Fig. 2b). Sex of the offspring in one out of three strains had a significant effect on F_1 fitness because daughters had lower relative fitness than sons (Table 2).

Insemination capacity of F_1 males

The insemination capacity of sons did not differ among various female mating treatments in any of the three strains. (*NDG* 11-$F_{4, 70} = 1.21$, $P = 0.31$; *TIW*1-ANOVA, $F_{4, 70} = 0.92$, $P = 0.45$; *PRUZ*-ANOVA, $F_{4, 67} = 2.47$, $P = 0.0524$; Table 3).

Table 2 Analysis of variance results on the relative fitness of F_1 males and females from monandrous and polyandrous mothers

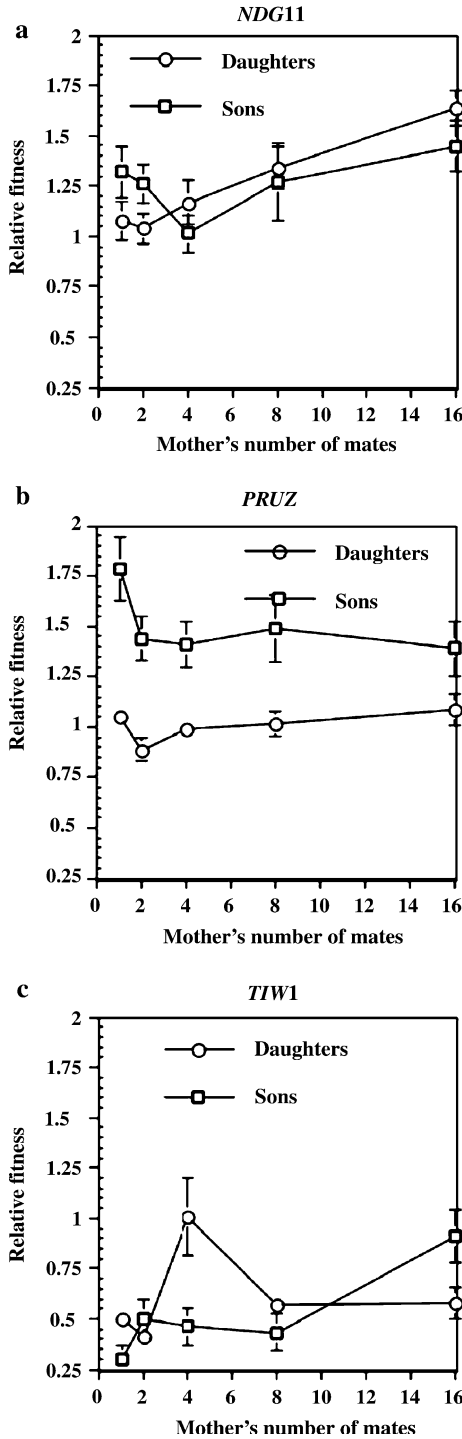| Strain	Source	*df*	SS	*F*	*P*
*NDG*11	Sex	1	0.004	0.01	0.89
	Number of mates	4	5.609	4.67	0.001
	Sex *X* number of mates	4	1.965	1.63	0.16
	Error	220	66.04		
*TIW*1	Sex	1	0.444	2.73	0.09
	Number of mates	4	6.418	6.79	<0.0001
	Sex *X* number of mates	4	4.175	6.41	<0.0001
	Error	210	34.157		
PRUZ	Sex	1	13.944	62.38	<0.0001
	Number of mates	4	1.694	1.89	0.11
	Sex *X* number of mates	4	1.157	1.29	0.27
	Error	217	48.500		

Fig. 2 Relative fitness of F$_1$ individuals from monandrous and polyandrous females (**a**) *NDG*11, (**b**) *PRUZ*, and (**c**) *TIW*1 (mean and standard error are shown)

F_1 males' partners' fecundity, F_2 adult production, and F_2 egg-to-adult viability

Egg production of partners of F_1 males, F_2 adult progeny production, and egg-to-adult viability did not differ significantly among the mating treatments in any of the three strains (Tables 3, 4).

F_1 female fecundity, F_2 adult production, and F_2 egg-to-adult viability

In *NDG*11, egg production of F_1 females differed significantly among the treatments (Tables 5, 6). F_1 females from mothers with 4 available partners had higher fecundity than F_1 females from mothers with two available partners (Tukey–Kramer HSD, $P < 0.05$, Table 5). However, this did not translate into a difference in F_2 adult progeny production and egg-to-adult viability (Tables 5, 6). The *TIW*1 strain also showed a significant among-treatment variation in fitness correlates of F_1 (Table 6). In particular, F_2 adult progeny production, but not fecundity or F_2 egg-to-adult viability of F_1 females was significantly different among treatments (Table 6). F_1 females from mothers with 16 available partners had higher F_2 adult progeny production than F_1 females from mothers with four available partners (Tukey–Kramer HSD, $P < 0.05$, Table 5). In *PRUZ*, both egg production of F_1 females as well as egg-to-adult viability differed significantly due to mothers' promiscuity, although F_2 adult progeny production was not influenced by mating treatment (Tables 5, 6). A Tukey–Kramer HSD test failed to reveal significant differences among treatments in egg production of F_1 females or in egg-to-adult viability.

Discussion

The present study demonstrated that different strains of the red flour beetle varied in the degree of polyandry as well as its fitness consequences. Where *NDG*11 and

Table 3 Insemination capacity, F_1 males' partners' fecundity, F_2 egg-to adult viability, and F_2 adult progeny of F_1 males

Strain	Mother's number of mates	Mean number of females inseminated (SE)	Mean number of eggs produced (SE)	Mean number of adult progeny produced (SE)	Mean egg-to-adult viability (SE)
*NDG*11	1	0.86 (0.21)	17.64 (2.61)	7.35 (2.07)	0.37 (0.07)
	2	1.26 (0.28)	20.35 (2.77)	11.38 (1.47)	0.57 (0.07)
	4	1.46 (0.21)	16.21 (2.89)	7.00 (1.83)	0.31 (0.08)
	8	1.60 (0.38)	18.14 (2.53)	7.07 (1.92)	0.31 (0.07)
	16	1.53 (0.19)	26.78 (2.94)	14.42 (1.82)	0.53 (0.05)
*TIW*1	1	0.93 (0.11)	8.84 (2.06)	4.30 (1.27)	0.55 (0.16)
	2	0.53 (0.13)	13.78 (1.80)	6.64 (1.09)	0.49 (0.05)
	4	0.86 (0.19)	12.00 (1.87)	5.23 (0.90)	0.40 (0.06)
	8	0.80 (0.32)	15.35 (1.66)	5.14 (0.73)	0.37 (0.04)
	16	0.53 (0.13)	16.73 (1.70)	4.26 (0.79)	0.28 (0.05)
PRUZ	1	1.86 (0.37)	18.88 (4.10)	8.88 (2.02)	0.50 (0.09)
	2	2.20 (0.29)	22.00 (3.52)	10.75 (2.88)	0.48 (0.12)
	4	1.93 (0.31)	16.83 (3.30)	9.75 (2.08)	0.57 (0.10)
	8	1.00 (0.21)	18.45 (3.70)	8.18 (2.33)	0.45 (0.10)
	16	1.08 (0.07)	19.9 (3.29)	10.10 (2.34)	0.50 (0.10)

SE Standard error

Table 4 Multivariate analysis of variance on effect of mating treatment on F_1 males' fitness correlates (F_1 males' partners' fecundity, F_2 adult progeny production, and F_2 egg-to-adult viability)

Factor	Wilk's λ	F_1^a	df	P	F_2^b	df	P
*NDG*11 sons	0.77	1.00	12	0.444			
F_1 males' partners' fecundity					1.52	4	0.210
F_2 adult progeny production					1.89	4	0.126
F_2 egg-to-adult viability					0.77	4	0.548
*TIW*1 sons	0.79	1.03	12	0.425			
F_1 males' partners' fecundity					1.02	4	0.402
F_2 adult progeny production					0.47	4	0.755
F_2 egg-to-adult viability					1.71	4	0.145
PRUZ sons	0.82	0.58	12	0.85			
F_1 males' partners' fecundity					0.30	4	0.872
F_2 adult progeny production					0.99	4	0.420
F_2 egg-to-adult viability					0.55	4	0.697

[a] Approximately F

[b] Univariate ANOVA F-test

Table 5 Fecundity, F_2 egg-to adult viability, and F_2 adult progeny production of F_1 females

Strain	Mother's number of mates	Mean number of eggs produced (SE)	Mean number of adult progeny (SE)	Mean egg-to-adult viability (SE)
*NDG*11	1	33.06 (6.33)	13.21 (3.51)	0.29 (0.06)
	2	19.38 (2.82)	8 (1.98)	0.35 (0.06)
	4	52.00 (6.38)	19.26 (3.11)	0.34 (0.04)
	8	35.66 (4.48)	14.58 (2.96)	0.37 (0.04)
	16	30.69 (7.30)	11.00 (3.41)	0.31 (0.07)
*TIW*1	1	34.00 (3.94)	14.50 (1.99)	0.41 (0.06)
	2	30.25 (3.61)	12.66 (2.00)	0.40 (0.05)
	4	19.33 (3.17)	11.50 (1.85)	0.59 (0.08)
	8	32.6 (4.13)	16.73 (2.42)	0.50 (0.05)
	16	39.23 (6.85)	22.00 (3.45)	0.57 (0.05)
PRUZ	1	13.06 (1.83)	8.53 (1.57)	0.62 (0.09)
	2	13.66 (1.23)	9.06 (1.25)	0.64 (0.06)
	4	14.57 (1.52)	6.71 (1.68)	0.40 (0.08)
	8	18.53 (1.85)	9.86 (1.53)	0.52 (0.07)
	16	19.40 (1.57)	10.93 (1.16)	0.54 (0.04)

SE standard error

PRUZ females mated with an average of 3–4 males within an hour, the majority of *TIW*1 females did not copulate at all, and those that mated only did so once in the 1 h observation period. In one population, *PRUZ*, we observed only costs of polyandry, in another, *NDG*11, only benefits of polyandry, while in *TIW*1, we detected both costs and benefits.

The three test strains differed significantly in the extent of polyandry (Table 1). *PRUZ* and *NDG*11 females remated readily, whereas *TIW*1 females appeared to be very resistant to remating. This has been previously documented in beetles of *TIW* background by Nilsson et al. (2002). Although we did not observe multiple mating by *TIW*1 females within the 1hr observation period, our previous study showed that *TIW*1 females did mate with multiple males when the experimental timeframe was longer (2 days, Pai and Yan 2002b). The experiments measuring offspring production

Table 6 Multivariate analysis of variance on effect of mating treatment on F_1 females' fitness correlates (F_1 female fecundity, F_2 adult progeny production, and F_2 egg-to-adult viability)

Factor	Wilk's λ	F_1^a	df	P	F_2^b	df	P
*NDG*11 daughters	0.67	2.12	12	0.017			
F_1 female fecundity					3.96	4	0.006
F_2 adult progeny production					2.05	4	0.097
F_2 egg-to-adult viability					0.83	4	0.510
*TIW*1 daughters	0.69	1.92	12	0.035			
F_1 female fecundity					2.42	4	0.057
F_2 adult progeny production					2.77	4	0.034
F_2 egg-to-adult viability					1.90	4	0.121
PRUZ daughters	0.67	1.90	12	0.037			
F_1 female fecundity					3.36	4	0.015
F_2 adult progeny production					0.51	4	0.721
F_2 egg-to-adult viability					2.95	4	0.027

[a] Approximately F

[b] Univariate ANOVA F-test

and other fitness components had a sufficiently long timeframe (10 days), so that *TIW*1 females should have mated with multiple males.

Increase in offspring production is a direct benefit of polyandry and has been observed in many different species (Ridley 1988; Arnqvist and Nilsson 2000). We found that the direct consequences of polyandry varied across our study populations because we detected direct effects of polyandry in only two of three strains (Fig. 1). In both cases, females mated with a single male produced more progeny than females exposed to multiple partners. In another highly polyandrous red flour beetle strain, c*SM*, we found no effect of female mating with multiple virgin males on both short-term (Pai and Yan 2002a) or long-term (Pai and Yan 2003) offspring production. The reduction in progeny production by polyandrous females of the *TIW*1 and *PRUZ* strains seen in the present study may be caused by decreased egg production, decreased egg-to-adult survival, or increased cannibalism on eggs. In the first 10 days, the experiments with more adult males were relatively more crowded. If the strains are highly cannibalistic, increase in adult male beetle density could lead to increased consumption of eggs by adults, but the beetle cultures were maintained with optimal supplies of food and cannibalism by adults seems unlikely. Another possibility is that the higher number of males in treatments with multiple (2,4,8,16) males and the male biased sex ratio could have lead to increased sexual harassment to females which might be one of the causes for lower offspring production of polyandrous females (Drummond 1984). Careful tests controlling adult density in oviposition vials are required to examine the possible effects of population density on the direct consequences of polyandry. However, previous studies on other beetle species have reported results of polyandry that include lower hatching rate of eggs (leaf beetles, Orsetti and Rutowski 2003) and lower pre-adult survival (a bruchid beetle, Eady et al. 2001). Therefore, reduced egg-to-adult survival or reduced fecundity are more likely explanations for the lower offspring production of females in multiple male treatments than is cannibalism, especially in light of the optimal food conditions maintained in our experiments.

Polyandrous females may increase their fitness indirectly by improving their offspring's fitness through obtaining good genes, compatible genes, attractiveness genes, or enhancing genetic diversity (Eberhard 1996; Zeh and Zeh 1996; Jennions and Petrie 2000). There is extensive evidence for indirect benefits of polyandry (e.g., Brooker et al. 1990; Olsson et al. 1994, 1996; Stockley et al. 1993; Zeh and Zeh 1996; Baer and Schmid-Hempel 1999, 2001; Evans and Magurran 2000; Konior et al. 2001) including in the red flour beetle (Bernasconi and Keller 2001; Pai and Yan 2002a). In the present study, the indirect fitness consequences of polyandry also varied among beetle strains. In *PRUZ*, we did not detect any effect of polyandry on the fitness of F_1 males and females (Fig. 2b, Table 2). In *NDG*11, we found indication of indirect benefits of polyandry because F_1 from females with 16 partners had significantly higher fitness than F_1 from females with 1, 2, or 4 partners in the competitive fitness assay (Fig. 2a, Table 2). Similarly, in *TIW*1, we found strong evidence for indirect fitness benefits of polyandry because both sons and daughters of polyandrous females fared better in the competitive assay than offspring of monandrous females (Fig. 2c, Table 2). That is, both F_1 males and F_1 females benefited from their mother's promiscuous mating. In our previous study with the c*SM* strain of this beetle, we found that F_1 males from polyandrous mothers had higher fitness whereas F_1 females showed lower fitness than those from monandrous females (Pai and Yan 2002a).

It might be hypothesized that density effects and not polyandry cause the difference in offspring fitness among various treatments in the competitive fitness assay. Thus, higher density in treatments with larger number of adults (example 8 or 16 males) might cause lower resource availability to offspring, leading to larval competition, and transgenerational effects of density leading to fitness differences of offspring. However, this should result in offspring from low density environments (females with only one male) to have higher fitness than offspring from high density environments (females placed with multiple males) and not the pattern of offspring from high density environments having higher fitness seen in this study. In another independent study in the same species, Bernasconi and Keller (2001) examined polyandry and sons' reproductive success over three generations in an environment carefully controlled for density and showed that sons' reproductive success was affected by polyandry over several generations (F_1, F_2 and F_3). The above indicate that in fact the fitness difference of offspring from monandrous and polyandrous mothers is a real one and not due to density effects.

The aforementioned study with the c*SM* strain also found that sons of polyandrous females were more successful in inseminating females than sons of monandrous females (Pai and Yan 2002a). In this study, we found that sons of polyandrous mothers did not inseminate significantly more females than did sons of monandrous mothers in any of the three strains (Table 3). The lack of difference among F_1 males from polyandrous and monandrous *PRUZ* females is not surprising because there was no detectable difference in their relative fitness. On the other hand, the lack of difference in insemination capacity among F_1 males from polyandrous and monandrous mothers in *TIW*1 and *NDG*11, in spite of a difference in their fitness, was unexpected. The most likely explanation for a lack of effect of polyandry on sons' insemination capacity in *TIW*1 is female reluctance to mate. As discussed above, *TIW* females are highly resistant to mating (Nilsson et al. 2002 and this study). It is quite likely that the 30 min time frame used in this study to test F_1 male insemination capacity was not long enough to detect a difference in their ability to

inseminate available females. Another possibility is that male mating behavior is different in the presence of rival males (competitive fitness assays, Table 3) compared to when there were no rival males (insemination assay, Table 4).

In two strains (*NDG*11 and *PRUZ*), F_1 female fecundity appeared to vary with mothers' promiscuous mating, but this did not lead to an increase in the number of adult progeny. It is thus unclear if polyandry confers indirect benefits through viability genes (Tables 5, 6). These findings are different from the pattern seen in our earlier study of the c*SM* strain, in which we found that polyandry confers viability benefits because F_1 males and females from polyandrous mothers produced F_2 with higher egg-to-adult survival (Pai and Yan 2002a). The results from experiments with *NDG*11 and *TIW*1 examining individual fitness components (insemination capacity of sons, fecundity, F_2 egg-to adult viability, F_2 adult progeny production) in a low competition environment failed to show any general patterns with respect to polyandry (Tables 3–6). Thus, the specific mechanism through which F_1 fitness was enhanced in *TIW*1 and *NDG*11 in the competitive assays needs to be determined.

Variation in polyandry and its fitness consequences shown in this study is possibly because of genetic differences among the populations, genetic drift, selection, or a combination of these factors. Our results are most consistent with the hypothesis that divergence among populations caused by genetic drift and/or sexual selection led to either cooperative or antagonistic co-evolution between the sexes.

Male-female co-evolution can be either antagonistic or cooperative. Sexual selection can create differences among populations and may create population divergence in two ways. First, if female reproductive behavior is evolving in response to indirect (genetic) benefits from multiple mating, then it may lead to co-evolution of male signals and female receptors such that these signals and receptors are different from other populations (Clark et al. 1999; Andrés and Arnqvist 2001). In this scenario of divergence shaped by sexual selection, male–female co-evolution is cooperative. In contrast, the second type of divergence shaped by sexual selection is through sexual conflict. Male and female reproductive interests are different, and such a conflict of interests may lead to antagonistic co-evolution between the sexes (Rice 1996; Parker and Partridge 1998; Holland and Rice 1999; Arnqvist et al. 2000; Andrés and Arnqvist 2001; Hosken et al. 2001; Gavrilets et al. 2001; Arnqvist and Rowe 2002). We hypothesize that populations of red flour beetles may differ in their pattern of male–female co-evolution. Some populations may exhibit cooperative co-evolution and others may exhibit antagonistic co-evolution, depending on the genetic background of the beetles and the environmental conditions. For example, *PRUZ* females showed only costs of polyandry, suggesting antagonistic co-evolution between the sexes. On the other hand, *NDG*11 showed only benefits and no costs to polyandry; this is consistent with the cooperative model of male-female co-evolution. Thus, these results suggest contrasting patterns of male–female co-evolution in different populations. In the guppy, *Poecilia reticulata* differences in ecology of populations resulted in differences in male- female interactions among various populations (Magurran and Seghers 1994) which might be one of the explanations for the differences we observe in flour beetle populations in this study. This hypothesis should be tested rigorously in future studies.

A number of examples have been documented in the literature on the variation in traits associated with reproduction among populations (e.g., Boake and Wade 1984; Endler and Houde 1995; Uy and Borgia 2000; Arnaud et al. 2001; Kwiatkowski and Sullivan 2002) and the fitness consequences of mating behavior (e.g., Gilburn and

Day 1994; Lesna and Sabelis 1999). Our results strongly suggest that generalizations about the fitness effects of polyandry should be made with caution.

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