Supplementary material

Study species – The Indian meal moth, Plodia interpunctella

P. interpunctella is a relatively short lived polygamous species with a ‘scramble competition’ mating system, in which both sexes have relatively low mating rates [1,2]. Mating begins with females advertising their position and receptivity to males through pheromones [3]. Attracted males then perform a courtship display consisting of rapidly fanning their wings and producing ultrasonic pulses [4]. Importantly, mating will not occur unless a female accepts a male by raising her abdomen which allows copulation to begin (J. Parrett, personal observation). There is no evidence that males have traits which directly harm females during copulation, however, occasionally males will harass unreceptive females for copulations (J. Parrett, personal observation), if and how harmful this is to females is unknown. Additionally, the ejaculate of *P. interpunctella* contain both fertile and non-fertile sperm, the latter may be involved in conflict over mating rates and act to decrease female remating [2,5], reduced female remating is likely to cause indirect costs to females by decreasing the genetic diversity of offspring. *P. interpunctella* represents a model species in which sexual selection can be broadly defined to consist of female choice and sperm competition. Furthermore, there is evidence of intra-locus sexual conflict in a number of shared life history traits [6] and the potential for inter-locus sexual conflict.

Supplementary method

Stock population

A large outbred stock population of *Plodia interpunctella* was cultured from multiple laboratory strains. Larvae were reared on a standard diet of bran middlings, yeast and glycerol at a ratio of 10:1:1, and all life stages were maintained at 27°C with a 12:12

light:dark cycle. The stock population was maintained at an approximate even sex ratio with non-overlapping generations and experimental evolution populations established after six generations.

Experimental evolution populations

Sixteen replicated populations were established from the outbred stock population following similar protocols outlined in Ingleby et al [2]. There were two treatments which altered the strength of sexual selection by manipulating the adult sex ratio; strong sexual selection within male biased populations (3:1 males: female; MB) and weak sexual selection within female biased populations (1:3; male: females; FB), each treatment replicated eight times. 120 adults at the stated adult sex ratio were used to found each population and subsequently establish the next generation. Similar designs with sexual selection manipulated by altering sex ratios have been used in previous studies and Lumley et al. [7] demonstrated that the heterozygosity of populations with similar effective population sizes used in this experiment were unaffected.

Populations were maintained by separating 5th instar larvae according to sex (the testes of male 5th instar larvae are clearly visible through the body wall) to ensure virginity, after eclosion all virgin adults were placed into egg collecting pots and after 72hrs eggs were collected which established the next generation (Figure S1). To maintain similar larval conditions approximately equal numbers of eggs were used from both MB and FB treatments. Larvae were reared on the standard diet *ad libitum* to remove any potential food competition between larvae. Once it became possible to sex larvae, collections were performed three times over a week in order to gain moths to both maintain populations and to be used in fitness assays (see below). At stable and slightly increased temperatures a surplus of larvae were collected, however, at increased temperatures the number of larvae decreased. Larvae were allocated at a ratio of 3:1 to maintain populations and experimental fitness assays, respectively. Our experimental design ensured a random representation of eggs, larvae and adults (which is proportional to the reproductive success of both males and females from the previous generation) in both maintenance of populations and those used during fitness assays.

The sixteen populations (8-MB and 8-FB) were randomly assigned to one of two experiments, creating four replicate populations for each sex ratio within each experiment. The two temperature experiments were assessing the fitness outcomes of sexual selection in stable or increasing temperatures. Populations assigned to the stable temperature experiment were maintained at 27°C throughout the entire experiment. Populations assigned to the increasing temperature experiment began the experiment at 27°C and the temperature was increased by 2°C every other generation until all populations became extinct. Temperatures were maintained using ten incubators made from expanded polystyrene boxes and heat mats each controlled by a thermostat. Using data loggers the temperature of each incubator was recorded every 10 minutes to ensure correct temperatures were achieved and maintained ± 0.5°C. To remove any incubator effect and reduce pseudoreplication every week incubators were randomly reassigned to stable or increasing temperature experiments. Additionally, populations and experimental moths were moved between incubators of the same temperature experiment daily.

Fitness assays

From each population and every generation (F_3 onwards) 5^{th} instar larvae were separated by sex to ensure virginity (Figure S1). Only virgin adults <24hrs old were used during fitness assays. Females were randomly assigned to a mating system and paired with either a single male (enforced monogamy) or three males (polyandry) and housed in 30ml vials for the duration of their lives. Fecundity was determined every 48hrs by moving all surviving moths to a new vial and counting eggs from the old vial under magnification. Offspring fitness was determined by estimating proportion egg to adult survival. A subset of 20 eggs were taken from each female and placed on an excess of food, if less than 20 eggs were laid all eggs were used. The number of emerged adults were counted and sexed after 6 weeks. The development time (egg to adult) of *P. interpunctella* is approximately 4 weeks [6], pots were also checked for any pupae not yet eclosed (which were never found), therefore all moths which survived to adulthood were recorded. The longevity of all the moths used during fitness assays were recorded. To provide insight into female reproductive strategy the number of eggs laid after 96 hours as a proportion of total fecundity was calculated for each female. In total the lifetime fecundity of 1796 females were determined (937 and 859 from the stable and increasing temperature experiments, respectively), of these 1759 females laid eggs and proportion offspring egg to adult survival recorded (923 and 836 from the stable and increasing temperature experiments, respectively).

Statistical analysis

All statistical analysis was carried out using R statistical software version 3.3.0 [8]. Model simplification was performed by removing non-significant terms until a minimal adequate model was reached and residuals checked for heteroscedasticity. All analysis was performed using mixed effects models with the R package *lme4* [9], with population as a random effect to account for variance from repeated measures within and across generations. The analyses were identical between temperature experiments, unless stated otherwise.

Population persistence to increasing temperatures was analysed using the total number of adults available to found the next generation and was always at the given sex ratio of the population (MB or FB). Moths used to maintain population gives an indication of the general productivity of the entire population. Only populations from the increasing temperature experiment were analysed. To test for any differences between population survival to increasing temperature and the strength of sexual selection experienced by the population a generalised linear mixed effect model with Poisson error structure was fitted. The total number of moths used to maintain the population was our response variable, population was included as a random effect and the explanatory variables were generation as a factor and sexual selection treatment (MB or FB) including their interaction term.

Generalised linear mixed effects models with Poisson error structure were fitted to fecundity data. To analyse proportion offspring survival, proportion of total eggs laid after 96hrs generalised linear mixed effects models with binomial error structures were fitted. In all models the explanatory variables were sexual selection treatment, mating system treatment and generation as a factor including all interaction terms. Population was used as a random effect to account for repeated measures within and between generations. A further observation level random effect was included in all models to account for overdispersion. Due to extremely low proportion offspring survival in generation F_8 within the increasing temperature experiment the full offspring survival model failed to converge. Similarly, the model used to analyse proportion eggs laid failed to converge using the full model and caused by extremely high proportion eggs laid during generation F_8 at increased temperatures. Therefore analysis was performed with offspring survival and egg laying rate data from generations $F_{3\text{-}7}$ from increasing temperature experiments, all other analyses was performed on full data sets from generations $F_{3\text{-}8}$.

Male and female longevity were analysed with log transformed values to improve the heteroscedasity of residuals and were used as our response variables in general linear mixed effects models. Sexual selection treatment, mating system treatment and generation as a factor were again used as explanatory variables and population used as a random effect. Due to potential pseudoreplication of male longevity from polyandrous mating system treatments, an extra random effect of vial number was included when analysing male longevity.

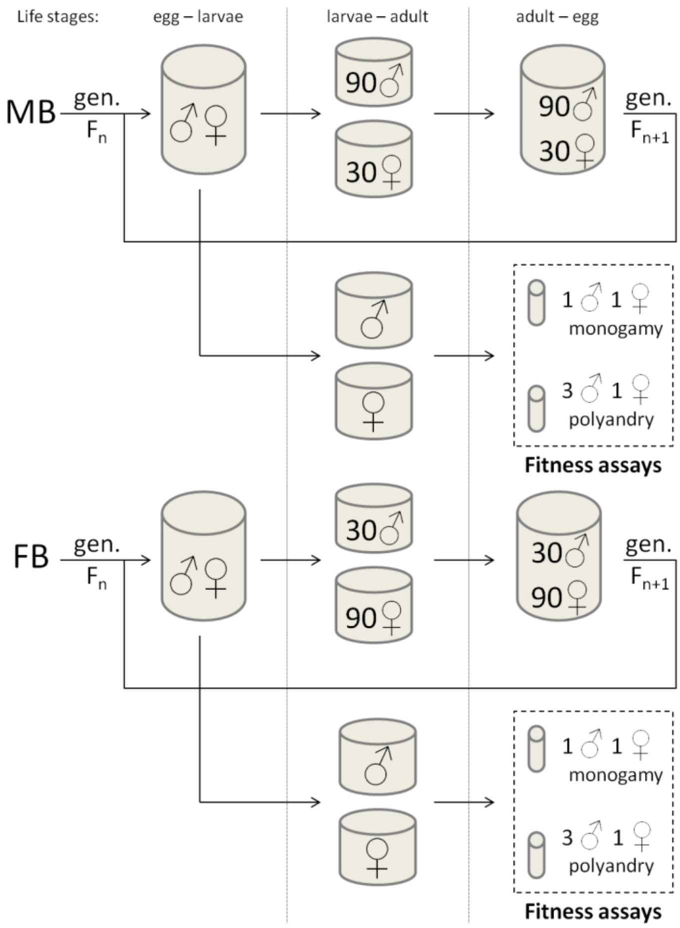

Figure S1. Experimental evolution protocols. Schematic of experimental design showing the maintenance of both strong (male biased; MB) and weak (female biased; FB) sexual selection treatments. The dashed boxes show that during fitness assays moths from both MB and FB populations were placed in monogamous and polyandrous mating systems, which removed and allowed sexual selection to act on individuals, respectively. Experimental design ensured a random representation of eggs, larvae and adults (which is proportional to the reproductive success of both males and females from the previous generation) in both maintenance of populations and those used during fitness assays.

References

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Supplementary results

Table S1. Results from fitting a generalised linear mixed effects model with Poisson error structure to predict the survival of populations from the increasing temperature experiment. The asterisk * indicates an interaction term between factors.

Source of variation	Population survival		
	χ^2	df	*p*
Sexual selection	0.00	1	0.989
Generation	2291.6	1	< 0.001
Sexual selection * Generation (as factor)	12.27	1	0.140

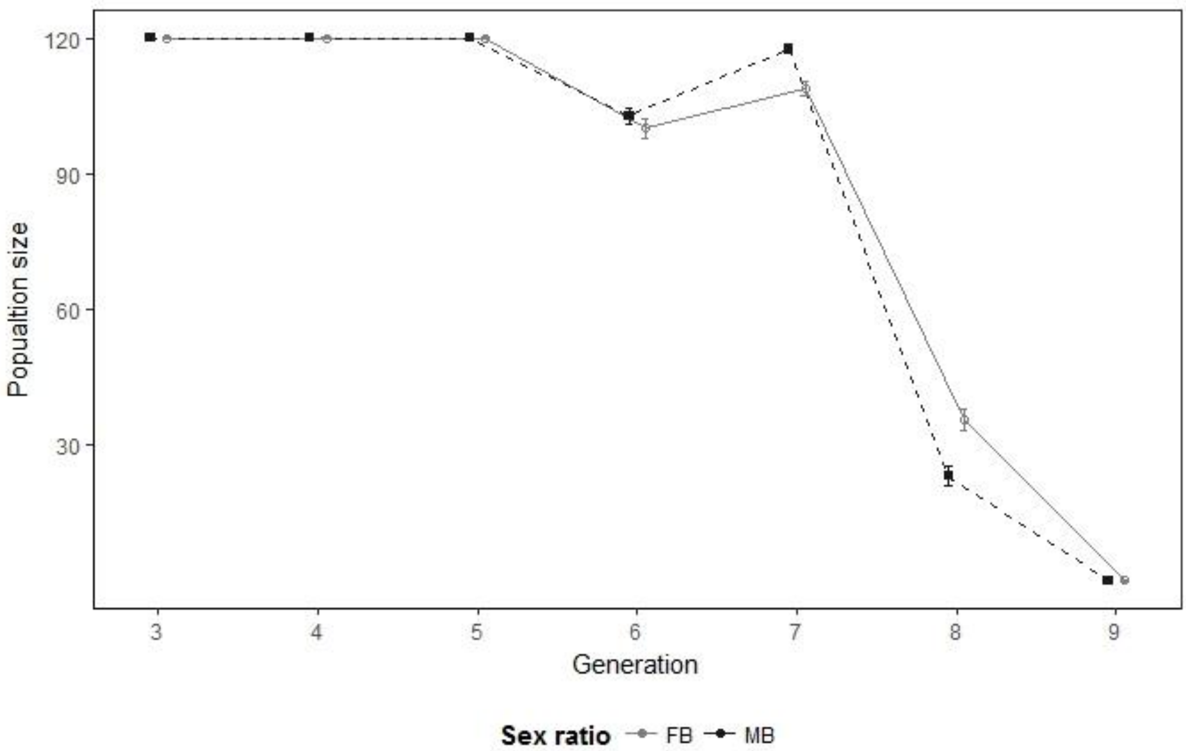

Figure S2. Survival of female biased (FB: light grey hollow circles and solid line) and male biased (MB: dark grey solid squares and dashed line) populations from the increasing temperature experiment across $F_{3\text{-}9}$. Points are mean (± SE) total number of adults used at each generation to maintain populations.

Table S2. Results from fitting mixed effects model to predict proportion offspring survival, fecundity, log transformed female longevity, log transformed male longevity and the proportion of total eggs laid after 96 hours from the increasing temperature experiment. The analyses of proportion offspring survival and the proportion of total eggs laid after 96 hours was performed on data from $F_{3\text{-}7}$ only. The asterisk * indicates an interaction term between factors.

Source of variation	Proportion offspring survival			Fecundity		
	χ^2	df	*p*	χ^2	df	*p*
Generation (as factor)	220.25	1	<0.001	-	-	-
Mating system	-	-	-	-	-	-
Sexual selection	-	-	-	-	-	-
Mating system * Generation (as factor)	4.21	1	0.378	13.08	1	0.023
Sexual selection * Generation (as factor)	3.25	1	0.517	8.55	1	0.129
Sexual selection * Mating system	6.20	1	0.013	3.88	1	0.049
Sexual selection * Mating system * Generation (as factor)	5.25	1	0.263	2.78	1	0.734

Source of variation	log (female longevity)			log (male longevity)		
	χ^2	df	*p*	χ^2	df	*p*
Generation (as factor)	250.49	1	<0.001	-	-	-
Mating system	39.40	1	<0.001	5.65	1	0.018
Sexual selection	13.13	1	<0.001	-	-	-
Mating system * Generation (as factor)	10.34	1	0.066	6.34	1	0.275
Sexual selection * Generation (as factor)	6.38	1	0.271	25.74	1	<0.001
Sexual selection * Mating system	0.00	1	0.980	0.11	1	0.741
Sexual selection * Mating system * Generation (as factor)	8.47	1	0.132	3.06	1	0.691

Source of variation	Proportion eggs laid in 96 hours		
	χ^2	df	*p*
Generation (as factor)	-	-	-
Mating system	-	-	-
Sexual selection	19.84	1	<0.001
Mating system * Generation (as factor)	11.35	1	0.023
Sexual selection * Generation (as factor)	2.79	1	0.593
Sexual selection * Mating system	0.19	1	0.661
Sexual selection * Mating system * Generation (as factor)	7.92	1	0.095

Table S3. Results from fitting mixed effects model to predict proportion offspring survival, fecundity, log transformed female longevity, log transformed male longevity and the proportion of total eggs laid after 96 hours from the stable temperature experiment from F $_{3\text{-}8}$. The asterisk * indicates an interaction term between factors.

Source of variation	Proportion offspring survival			Fecundity		
	χ^2	df	*p*	χ^2	df	*p*
Generation (as factor)	-	-	-	-	-	-
Mating system	5.91	-	0.015	-	-	-
Sexual selection	-	-	-	0.01	1	0.952
Mating system * Generation (as factor)	8.83	-	0.116	14.75	1	0.012
Sexual selection * Generation (as factor)	26.47	1	<0.001	2.45	1	0.784
Sexual selection * Mating system	0.01	1	0.936	0.19	1	0.666
Sexual selection * Mating system * Generation (as factor)	4.76	1	0.446	5.71	1	0.336

Source of variation	log (female longevity)			log (male longevity)		
	χ^2	df	*p*	χ^2	df	*p*
Generation (as factor)	161.58	1	<0.001	-	-	-
Mating system	69.56	1	<0.001	21.68	1	<0.001
Sexual selection	0.36	1	0.546	-	-	-
Mating system * Generation (as factor)	4.92	1	0.426	3.82	1	0.576
Sexual selection * Generation (as factor)	2.22	1	0.818	21.43	1	<0.001
Sexual selection * Mating system	3.09	1	0.079	0.06	1	0.800
Sexual selection * Mating system * Generation (as factor)	2.73	1	0.742	4.22	1	0.518

Source of variation	Proportion eggs laid in 96 hours		
	χ^2	df	*p*
Generation (as factor)	112.25	1	<0.001
Mating system	33.64	1	<0.001
Sexual selection	0.07	1	0.792
Mating system * Generation (as factor)	5.35	1	0.375
Sexual selection * Generation (as factor)	2.32	1	0.804
Sexual selection * Mating system	0.12	1	0.728
Sexual selection * Mating system * Generation (as factor)	6.91	1	0.227

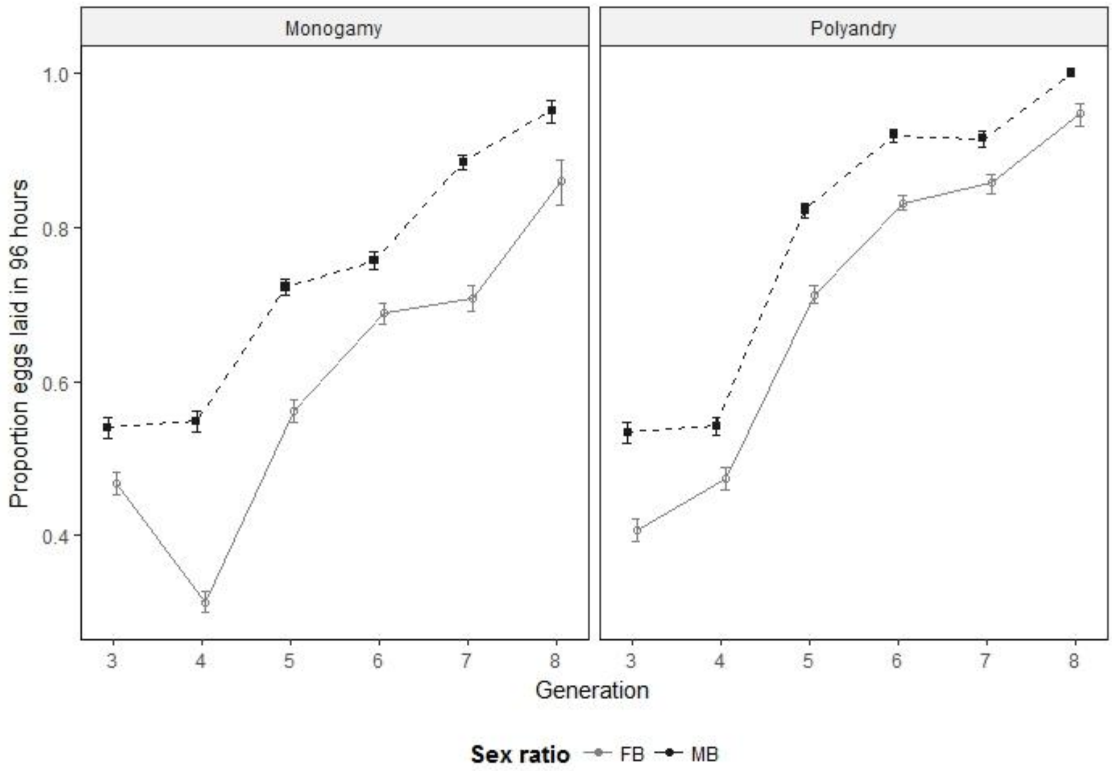

Figure S3. Comparison of the proportion of total eggs laid after 96 hrs from the increasing temperature experiment across $F_{3\text{-}8}$. Weak sexual selection (FB) is indicated by light grey hollow circles and solid lines and strong sexual selection (MB) indicated by dark grey solid squares and dashed lines. Males and females placed within monogamous or polyandrous mating systems during fitness assays are shown in the left and right panels, respectively. Error bars indicate 95% confidence intervals.

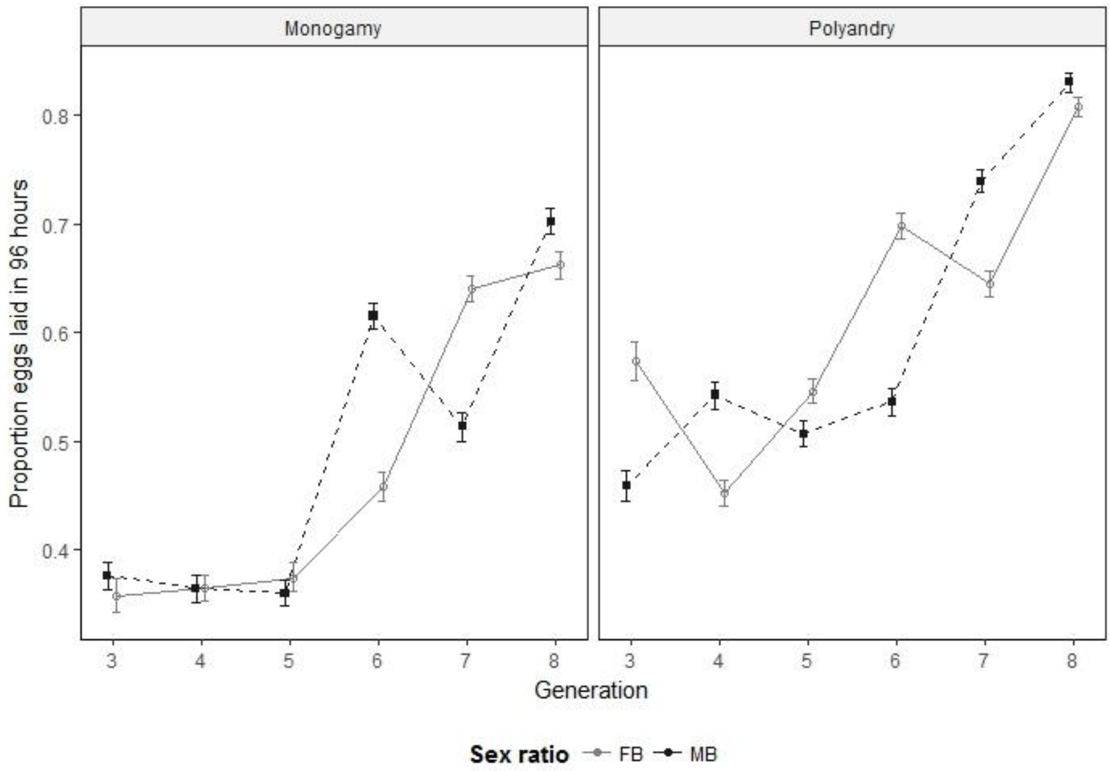

Figure S4. Comparison of the proportion of total eggs laid after 96 hrs from the stable temperature experiment across $F_{3\text{-}8}$. Weak sexual selection (FB) is indicated by light grey hollow circles and solid lines and strong sexual selection (MB) indicated by dark grey solid squares and dashed lines. Males and females placed within monogamous or polyandrous mating systems during fitness assays are shown in the left and right panels, respectively. Error bars indicate 95% confidence intervals.