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Supplemental Data

Testing for Direct and Indirect Effects of Mate Choice by Manipulating Female Choosiness

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Supplemental Results

The efficacy of female choice treatments. We conducted two distinct sets of experiments to verify that our experimental manipulations of female resistance ability had the intended effects. First, to test for effects on non-random mating by females, we first used phenotypic manipulation to experimentally create low-persistence males by simply ablating one fore tibia and one hind tibia of each male under light CO_2 anaesthesia. Since males use their fore legs to grasp females and their hind legs as support during mating attempts, our phenotypic manipulation compromised male ability to maintain an appropriate mating position during premating interactions with females and hence made males less persistent. We note that our manipulation did not noticeably reduce male viability and low-persistence males showed only a moderately depressed mating success (see below). We then introduced two virgin females (both from either of the five female choice treatment groups) to four virgin males (two low-persistence and two normal males) in a 30 mm ø Petri dish, and recorded all matings during 60 minutes (N = 10 such replicates per female choice treatment). All individuals were of 24 - 60 hours adult age and low-persistence males, as well as females, were at least 18 hours post-manipulation at the start of the experiments. Our

female choice treatment affected the number of matings observed ($F_{4,45}$ = 5.81, P < 0.001) (planned post-hoc comparisons; A: P = 0.556; B: P = 0.020; C: P = 0.630; D: P = 0.001): females with elevated resistance efficiency showed the lowest number of matings and those with reduced resistance efficiency the highest (mean number of matings per replicate ± SE: 1.6 ± 0.27, 1.8 ± 0.20, 3.1 ± 0.28, 2.1 ± 0.28, 1.9 ± 0.18; for females in group I-V, respectively). Our primary aim with the phenotypic engineering, however, was to alter the degree to which females bias matings in favour of persistent males. Overall, matings were indeed biased in favour of normal males over low-persistence males (76 and 29 observed matings, respectively, in total; χ^2_1 = 21.04, P < 0.001). More importantly, our experimental manipulation of female choice had the intended effect on female mating preferences: female choice treatment affected the proportion of matings that involved low-persistence males in the predicted direction (generalized linear model with binomial error and a logit link function; χ^2_4 = 11.62, P = 0.020) (planned post-hoc comparisons; A: P = 0.754; B: P = 0.024; C: P = 0.792; D: P < 0.001). Females with elevated resistance efficiency showed the lowest such proportion and those with reduced resistance efficiency the highest (mean proportion across replicates: 0.09, 0.13, 0.47, 0.21, 0.18; for females in group I-V, respectively). We predicted that the phenotypic effects of our choice manipulation would show a linearly increasing trend, rather than a non-linear response, from reduced over control groups to elevated choice. This was explicitly tested by assessing the first and second degree polynomial contrasts of the data above. These analyses supported a linear (number of

matings: $F_{1,47}$ = 19.46, P < 0.001; proportion of matings with low-persistence males $F_{1,46}$ = 10.64, P = 0.002) but not a non-linear (number of matings: $F_{1,47}$ = 3.60, P = 0.064; proportion of matings with low-persistence males $F_{1,46}$ = 1.86, P = 0.179) pattern of response to our treatments. A linear reponse pattern accounted for 84% and 83% of the variance between groups, whereas a non-linear reponse pattern accounted for only 15% and 14% (for number of matings and proportion of matings with low-persistence males, respectively).

Second, to additionally assess effects on copulation duration, we generated 20 virgin females for each of the treatment groups I-IV. Each female was then placed in a 30 mm ø Petri dish with 2 virgin normal stock males and observed closely for 30 minutes. We recorded the occurrence of mating and copulation duration. As in the previous experiment, there was a significant effect of treatment on female mating rate (likelihood ratio test; χ^2_3 = 9.07, P = 0.028). Again, the lowest mating rate was observed among females with elevated resistance efficiency (20%) and the highest among females with reduced resistance efficiency (60%) (planned post-hoc comparisons; A: P = 0.009; B: P = 0.341; C: P = 0.341; D: P = 0.009). Our treatment also significantly affected copulation duration (ANOVA of log-transformed copulation duration: $F_{3, 33}$ = 3.74, P = 0.020), such that females with a reduced resistance efficiency on average copulated for longer (mean ± SE; 87.0 sec. ± 18.1) than did females from the other three groups (49.0 sec. ± 14.23, 47.5 sec. ± 5.5 and 41.7 sec. ± 6.73, for

treatments I, II and IV, respectively) (planned post-hoc comparisons; A: P = 0.985; B: P = 0.011; C: P = 0.443; D: P = 0.127).

In sum, these experiments showed that (i) mating bias against low-persistence males was more pronounced, (ii) mating rate was lower and (iii) copulation duration was shorter in females with elevated resistance efficiency compared to those with reduced resistance efficiency.

The effect of irradiation on male fertility. We sterilised males by exposing them to a dose of 100Gy from a cesium-137 source at the division of Biomedical Radiation Sciences, Uppsala University. This sterile male technique has been shown to cause sterility in males while not compromising male copulation ability and sperm competitive ability in *C. chinensis* [1] as well as in a closely related seed beetle species [2]-[3]. We nevertheless conducted fertility assays to verify that our irradiation treatment caused full and lasting sterility among males used in the F1 fitness assays. In each replicate (N = 4), four virgin irradiated males were placed with four virgin stock females and 10 g of adzuki beans in 60 mm ø petri dishes. After 24 hrs, males were transferred to a new such set of virgin females. After 48 hrs, this procedure was repeated once again and males were then left with the females for their entire lifetime. Thus, each set of four irradiated males were kept with three groups of four virgin females in succession. All petri dishes were checked for hatched offspring and unhatched eggs after 35 days. Each set of four females laid on average 155.5 (SE = 11.8) eggs. Across the three

successive sets of females, the average proportion of these eggs that resulted in hatched offspring was 0%, 0.4%, 0.4% and 0.3%. Thus, these assays show that our treatment effectively induced lasting sterility in males.

Supplemental Experimental Procedures

Phenotypic engineering. Phenotypic engineering is common in various domains of evolutionary biology [4] and has two distinct advantages. First, because direct manipulation allows an extension of the range of phenotypic values, it increases the statistical power of tests for selection [4, 5]. This is particularly important when seeking to detect weak selection and the method is unbiassed when fitness functions are approximately linear [4]. Although the use of extreme phenotypic values often makes this approach less useful for precise estimations of the shape of non-linear fitness functions in natural populations, this fact does not compromise its utility when the principal goal of a study is to compare the relative strength of different types of selection. As long as the shapes of the fitness functions are similar across different types of selection, studying extreme phenotypes will be informative of the relative strength of selection [4]. In effect, phenotypic engineering can be seen as a method that allows the estimation of distinct components of fitness of mutant phenotypes, the origin of which is simulated by the manipulation [5]. The second, and most important, advantage stems from the fact that the focal phenotypic trait can be manipulated in isolation. Because of this, phenotypic engineering steers clear of

most of the problems with confounding effects that are involved when measuring phenotypic selection on correlated traits [4, 5]. In this sense, studies of phenotypic engineering are analogous to those employing genetic engineering (e.g., single gene knock-out mutants) to isolate the phenotypic effects of single genes.

Female fitness assays. Females were assembled in groups into replicate units, representing distinct subpopulations where environmental conditions and the population density match those experienced by this population since it was brought into the laboratory (see above), which also reproduces conditions encountered in the field [6]. Each replicate consisted of four or five virgin females (mean number of females per replicate ± SE; I: 4.54 ± 0.83; II: 4.8 ± 0.11; III: 5; IV: 5; and V: 5), sharing the same treatment, that were placed with an equal number of virgin 24 h old males from the stock population for life. Each such assembly of beetles were kept in a 90 mm ø Petri dish with 30 g of fresh organically cultivated Adzuki beans (~375 beans) that had been frozen prior to use. We note here that the number of beans in each replicate thus exceeded the total number eggs produced by females, thus preventing larval competition within replicates. We initiated a total of 74 replicate units across the five treatments (N = 13 – 17 per treatment) during a nine-day period. After 10-12 days, when all adults were dead and all larvae hatched, we inspected all beans and recorded the number of hatched and unhatched (infertile) eggs in each replicate unit. At the same time, 48 beans with eggs were selected from each replicate unit and

isolated individually (virgins hatching from these beans were later used in the F1 fitness assays – see below). Thirty-five days after the onset of each replicate, the number of adult offspring produced was recorded. These data were then used to generate four different components of fitness, all based on average values per female in each replicate: (i) lifetime fecundity (total number of eggs laid), (ii) lifetime offspring production (number of adult offspring emerged), (iii) hatching rate of eggs (number of hatched eggs / total number of eggs) and (iv) survival rate of larvae (number of adult offspring emerged / number of hatched eggs). We thus define “direct effects” as those involving effects on all components of female lifetime offspring production, noting that two of these (iii and iv) can in part be influenced by variance in the paternal genetic contribution to offspring.

F1 fitness assays. In order to test specifically for indirect selection, we assayed male and female fitness in the F1 progeny of experimental females. From each replicate unit (see above), we created two subreplicates of male reproductive fitness and two subreplicates of female fitness (see below) and the mean fitness in the two subreplicates was used for further analyses. Male reproductive fitness was assayed by allowing a set of focal males to compete with a set of sterilized stock males over mating and fertilization of eggs. In each subreplicate, five virgin males from singly inoculated beans from an experimental unit were placed for life with eight randomly selected stock males that had been sterilized by irradiation (see Supporting Information) and ten randomly selected virgin stock females in a 90 mm ø Petri dish provided with 30 g of Adzuki beans. Thirty-five days after the

initiation of these assays, all emerged adult offspring were recorded. Because eggs fertilized by stock males did not develop (see above), the number of offspring produced in these assays represents a direct measure of the lifetime reproductive fitness of focal males. This integrative assay thus amalgamates the pre- and post-copulatory success of focal males relative to stock males and includes components of both male-male competition and female choice. Female fitness was assayed by recording lifetime offspring production. In each subreplicate, five virgin focal females from an experimental unit were placed for life with 5 randomly selected stock males in a 90 mm ø Petri dish provided with 30 g of Adzuki beans. Thirty-five days after the initiation of these assays, all emerged adult offspring were recorded and taken as a measure of female lifetime reproductive success. In total, the assays of male and female F1 fitness were based on 69 436 produced F2 offspring.

Statistical analysis. To test for treatment effects on direct and indirect fitness components, we used conventional general linear models using female choice treatment as our factor. Because hatching date is known to be associated with environmentally based maternal effects that can be transmitted across generations in seed beetles [7, 8], we included date of the start of each replicate unit as a continuous covariate in our inferential models. However, we note here that inclusion of date in our models did in no case alter our ability or inability to reject null hypotheses regarding the effects of female choice treatments. Further, in no case did the interaction between treatment and date improve model fit to

data (partial *F* - tests, $P > 0.25$ in all cases). Residual distributions were not significantly different from normality for five of our inferential models (Shapiro-Wilk's test; $P > 0.3$ in four cases and $P > 0.05$ in one case) but showed positive and significant kurtosis in one case ($\gamma_2 = 1.48$; Shapiro-Wilk's test; $P < 0.05$). For this reason, we also evaluated all models by resampling tests, involving bootstrapping (5000 replicates) the residuals of the original models [9, 10]. For post-hoc testing, we used the following inferential path. Provided that the omnibus model showed a significant treatment effect, we performed four planned post-hoc comparisons: (A) a test between elevated choice against its control (I versus II); (B) a test between reduced choice and its control (III versus IV); (C) a test for differences between the three control groups (II, IV and V) and (D) a test between the two female choice treatments (I versus III).

Supplemental References

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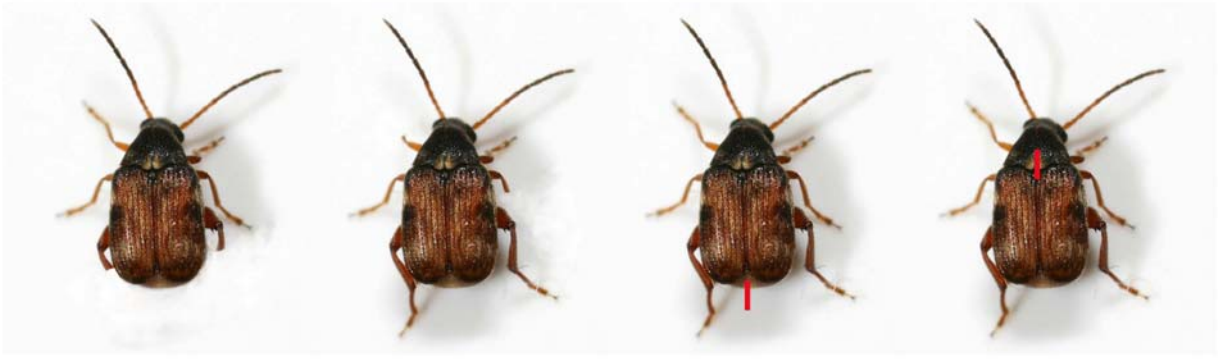
Figure S1



Lowered resistance ability

Control for lowered resistance ability

Elevated resistance ability

Control for elevated resistance ability

Figure S1: Schematic illustration of the phenotypic engineering used. Female resistance ability was reduced by ablating rear legs (used to thwart males attempting to mate) and evelated by attaching a prong to the rear abdominal plate (indicated by red rectangle). Behavioral observations confirm that these treatments had the intended effects on female ability to resist male mating attempts.