

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

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Summary

Despite a massive research effort, our understanding of the evolution of female mate choice remains incomplete [1, 2]. A central problem is that the predominating empirical research tradition has focused on male traits, yet the key question is whether female choice traits are maintained because of direct effects on female fitness or because of indirect genetic effects in offspring that may be associated with such traits. Here, we address this question by using a novel research strategy that employs experimental phenotypic manipulation of a female choice trait in an insect model system, the seed beetle *Callosobruchus chinensis* (Coleoptera: Bruchidae). We show that females with increased efficiency of choice enjoy strongly elevated fitness compared to females with reduced choice efficiency. In contrast, we found no effects of female choice efficiency on offspring fitness. Our results show that female choice is maintained by direct selection in females in this system, whereas indirect selection is relatively weak at most. We suggest that phenotypic engineering of female choice traits can greatly advance our ability to elucidate the relative importance of direct and indirect selection for the maintenance of female choice.

Results and Discussion

Males and females are often strikingly different, showing sexual dimorphism in behavior, morphology, and physiology. Many of these differences between the sexes are the result of sexual selection by female choice [1]. Female choice is not a trait per se but is a form of nonrandom mating that results from a wide variety of female choice traits, including not only behavioral but also morphological, physiological, and perceptual features of females [2]. Female choice traits (or female preference traits) all have the effect in common that they, directly or indirectly [3], cause females to be more likely to mate with some conspecific male phenotypes than others [4]. Although empirical research sometimes separates “active” or “direct” from “passive” or “indirect” female choice, we note that such a distinction lacks theoretical foundation [2–6]. There is a lack of empirical studies that measure different components of phenotypic selection on female choice traits [5, 6]. This deficiency is unfortunate because the critical difference between models of the evolution of female choice concerns the type of selection that acts on such traits. In particular, “good genes” models [7] predict that indirect selection favors efficient female choice whereas models involving direct effects on female fitness [8, 9] predict that direct selection maintains female choice. Although it is difficult to quantify natural

variation in female choice trait phenotypes [10, 11], selection can be studied by documenting the fitness effects of direct manipulation of phenotypic traits (phenotypic engineering) [12]. Following the pioneering tail length manipulation in male widow birds by Andersson in 1982 [13], phenotypic engineering has been widely used in studies of female mate choice. However, the trait under manipulation has invariably been the focal male trait rather than the female choice trait itself. These studies thus ask *whether* female choice occurs rather than *why*. Here, we use phenotypic engineering [12] to directly manipulate a female choice trait for the first time, and we compare the associated direct and indirect effects on female fitness in the Adzuki seed beetle, *Callosobruchus chinensis*.

The female choice trait that we focus on here, overt female resistance to courting or harassing males, is very widespread in nature and is known to bias matings toward male phenotypes that are more vigorous or persistent in a wide range of taxa [2]. This female choice trait has been suggested to be the result of both direct and indirect selection [14]. A large and outbred population of *C. chinensis* (Coleoptera: Bruchidae) was used to experimentally alter female choice phenotypes. We created the following five female choice treatments: (1) elevated female resistance efficiency; (2) a control for elevated resistance; (3) decreased female resistance efficiency; (4) a control for decreased resistance; and (5) a global unmanipulated control (see [Experimental Procedures](#) for details). Females with experimentally elevated/decreased resistance showed stronger/weaker female preference for vigorous males (see [Supplemental Data](#), available online).

To assess direct effects of our treatment, we assayed female fitness in replicated groups of females under conditions that these beetles normally experience (see [Supplemental Data](#)). Each replicate (n = 13–16 per treatment) consisted of four or five virgin females, sharing the same treatment, that were placed for life with an equal number of males. We then recorded the following four components of lifetime female fitness: fecundity, offspring production, hatching rate of eggs, and survival rate of larvae. To assess indirect effects, we used integrative assays to measure total lifetime fitness in male and female offspring of the experimental females (see [Supplemental Data](#)). The reproductive fitness of their sons was assayed by allowing a set of focal males to compete with a set of sterilized males over mating and fertilization while recording lifetime offspring production. We created two subreplicates per replicate. This assay thus measures the net pre- and postcopulatory success of focal males relative to a standard competitive background. The fitness of their daughters was assayed by recording lifetime offspring production in sets of females kept with males for life. Again, we created two subreplicates per replicate.

We found that female resistance efficiency had a strong direct effect on female fitness ([Table 1](#)). Most importantly, female lifetime fecundity and offspring production were elevated in females with increased resistance efficiency and depressed in females with decreased resistance efficiency, whereas the three control treatments were not significantly different and showed intermediate fitness (see [Figure 1](#)). The size of this effect was remarkably large. For example, the

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Table 1. The Effects of Experimentally Altered Female Choice Phenotypes on Female Fitness

Source	SS	df	F	p	p _r
Lifetime Fecundity					
FC	1773.6	4.67	10.5	<0.001	<0.001
D	173.3	1.67	4.1	0.047	0.051
Lifetime Offspring Production					
FC	1065.4	4.67	5.25	<0.001	0.002
D	19.5	1.67	0.39	0.537	0.629
Hatching Rate of Eggs					
FC	0.0515	4.67	0.88	0.480	0.485
D	0.0001	1.67	0.01	0.924	0.894
Survival Rate of Larvae					
FC	0.0283	4.67	1.76	0.148	0.146
D	0.0339	1.67	8.40	0.005	0.003
Reproductive Success of Sons					
FC	26647.9	4.67	2.22	0.076	0.080
D	68064.7	1.67	22.72	<0.001	<0.001
Offspring Production of Daughters					
FC	4562.6	4.66	0.81	0.524	0.517
D	7072.5	1.66	5.01	0.028	0.032

FC refers to effects of the female choice treatment, and the covariate D refers to effects due to the date at the start of each replicate. The first four models estimate direct effects on female fitness, and the last two models assess the effects on the reproductive success of offspring. All models were tested with conventional *F* tests (*p*) as well as with resampling tests (*p_r*).

lifetime offspring production of females with increased resistance efficiency was approximately 60% higher than that of females with decreased resistance efficiency. This difference in mean fitness corresponds to 1.5 times the average within-group standard deviation or a selection coefficient of *s* = 0.38. Previous studies of the Adzuki seed beetle suggest that direct selection for high female resistance efficiency in this system is due to a combination of decreased costs of multiple mating (i.e., a decreased mating rate; see Supplemental Data) [15, 16], less time and energy being spent on rejecting courting males [17], and reduced male interference during oviposition [18].

In contrast, phenotypic manipulation of female resistance efficiency had no significant effect on progeny fitness, either among female or male offspring of the manipulated females (Table 1). Treatment effects on the reproductive success of sons may appear sizeable, even if they were statistically nonsignificant, but we note that these indirect effects were not in the predicted direction: sons of females with decreased resistance efficiency actually had the highest mean reproductive success of all five treatment groups (see Figure 2). The lack of effects of the paternal genetic contribution to offspring was also evident from the fact that there were no significant effects of our female choice treatment on hatching rate of eggs or on juvenile survival (Table 1), both of which could in part be affected by variance in the paternal genetic contribution. In theory, our failure to detect significant indirect effects could be due to a depressed level of additive genetic variance for fitness-related traits in our population. However, four facts collectively imply that this is not the case. First, our study population was founded by a very large number of wild caught individuals and it has been kept at a large effective population

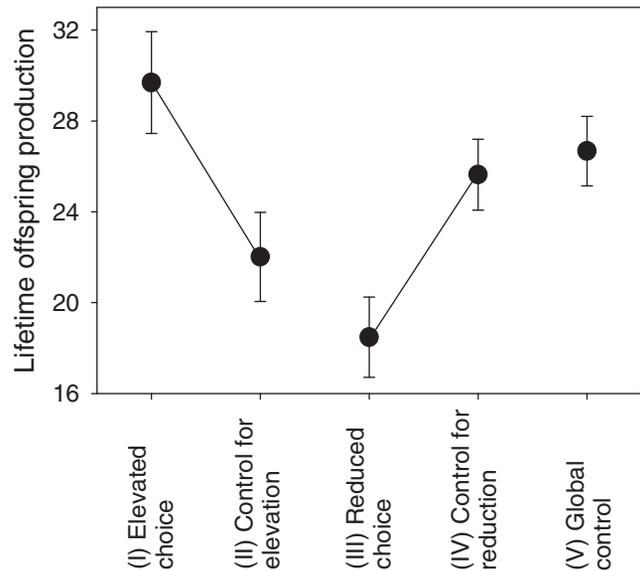


Figure 1. The Direct Effects of Experimentally Increased and Decreased Female Choice Efficiency on Female Lifetime Offspring Production

Given are mean (± SEM) offspring production per female of females subjected to the female choice treatments ($F_{4,67} = 5.25$, $p < 0.001$). Planned post-hoc comparisons were as follows (see Supplemental Results): A [(I and II): $F_{1,31} = 5.95$, $p = 0.021$; B [(III and IV): $F_{1,31} = 7.06$, $p = 0.012$; C [(II, IV, and V): $F_{2,50} = 1.77$, $p = 0.180$; and D [(I–III): $F_{1,31} = 27.70$, $p < 0.001$].

size in the laboratory ever since (see Experimental Procedures). Second, our measure of mean offspring fitness was repeatable across groups of females for sons (male offspring: correlation between the two subreplicates; $r = 0.46$, $p < 0.001$; partial correlation between the two subreplicates, accounting for covariance with date and effects of treatment; $r_{XY.Z1Z2} = 0.31$, $p = 0.009$; female offspring: $r = 0.11$, $p = 0.35$; $r_{XY.Z1Z2} = 0.07$, $p = 0.59$). Third, mean female lifetime offspring production per female group was significantly and negatively correlated with the mean fitness of their sons (male offspring: $r = -0.30$, $p = 0.011$; $r_{XY.Z1Z2} = -0.316$, $p = 0.010$; female offspring: $r = 0.04$, $p = 0.73$; $r_{XY.Z1Z2} = 0.09$, $p = 0.48$). The latter two observations are consistent with the presence of standing genetic variation in key fitness components in our study population. Fourth, and most importantly, several studies of experimental evolution [19, 20] and direct quantitative genetic estimations [21–23] have revealed sizeable amounts of standing additive genetic variation for fitness components in similar populations of seed beetles. For example, both female fecundity [22] and female resistance to remating [20] show additive genetic variation in laboratory populations of the Adzuki seed beetle founded by fewer individuals and kept at smaller population sizes than the one used here.

Our view of the evolution of female choice has perhaps grown somewhat less polarized during the last decade or so: most scholars now fully acknowledge that direct and indirect selection act simultaneously on female choice traits in most taxa [2]. A growing body of empirical data, based largely on comparing the progeny of males with different phenotypes, has shown that the potential for “good genes” effects are certainly at hand in many populations [24]. However, such studies are not conclusive simply because they focus on male characteristics rather than on selection in females: they do not directly address the net fitness effects of different

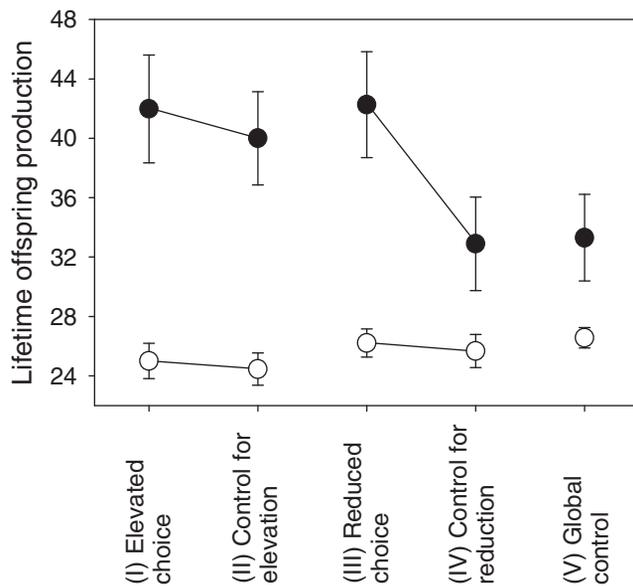


Figure 2. The Indirect Effects of Experimentally Increased and Decreased Female Choice Efficiency on Offspring Fitness

Given are mean (\pm SEM) lifetime offspring production per individual son (solid symbols; $F_{4,67} = 2.22$, $p = 0.076$) and daughter (open symbols; $F_{4,66} = 0.81$, $p = 0.524$) of the females subjected to the female choice treatments. Note that in two out of four cases the observed nonsignificant effects are in a direction opposite to that predicted.

expression of the female choice trait in question. This deficiency is aggravated by the fact that virtually all conceivable female choice traits have important effects other than causing nonrandom mating (they may, e.g., affect foraging success, absolute mating rate, ability to avoid predators, or amount of paternal care received) [25–28]. Studies that are restricted to measuring the effects of reproducing with males of different phenotypes on female fitness are inherently ignorant to such effects. Only comprehensive studies of phenotypic selection on female choice traits can include all relevant sources and forms of selection [5, 6]. One consequence of the lack of studies of selection on female choice traits is that the long-standing key question as to the relative importance of direct and indirect selection on female choice traits has remained largely unresolved. Theory suggests that indirect selection should be weak relative to direct selection on female choice traits [29], but integrative empirical estimates that directly quantify the two types of fitness effects have been absent. The main conclusion from our work is that indirect selection on female choice traits in this model system is very weak (at most) when compared with direct selection. The fact that our results are in line with a few recent studies of birds and insects comparing direct and indirect selection using less direct approaches [11, 27, 30] suggests that our findings are not specific to our model system or experimental setting. Thus, although all models for the evolution of female choice predict that indirect selection on female choice traits should often occur [2, 6, 14, 24], the available direct empirical data suggest that indirect selection on female choice traits is generally very weak when compared with direct selection. This implies that, in any given system, an observation of the potential for indirect genetic benefits is insufficient for suggesting that indirect selection is an important form of selection on the female choice trait in question [29]. This is especially true when

considering that the heritability of female choice traits may often be fairly low [11, 29] and standing genetic variation in fitness may frequently be sexually antagonistic [31–34]. Although sexually antagonistic selection may greatly promote the maintenance of genetic variation for fitness, the fact that “good genes” effects are then sex specific and do not provide equal benefits to sons and to daughters means that the strength of indirect selection, where additive genetic effects are averaged over the two sexes [29], will be considerably weakened or even nullified [34, 35]. Sex specificity of fitness effects is therefore of key importance when measuring indirect selection on female choice traits.

Our study shows that phenotypic engineering of female choice traits can provide very important insights to this field, in systems and situations where it is at all feasible. This experimental approach can yield much needed empirical data on the relative strength of direct and indirect selection on female choice traits, while avoiding many of the problems normally associated with estimating selection on such traits.

Experimental Procedures

Study Population and Mating Behavior

Bruchid beetles are economically important stored product pests in the tropics and populations of *Callosobruchus* seed beetles can reach very high densities in storages of dry beans [36]. Here, we used a population of the seed beetle *C. chinensis*, which was obtained from T. Miyatake (isC; Okayama University, Japan). This population was founded by more than 100 mated female beetles collected from a dry-bean storage site (Ishigaki City) by K. Kohno in 1997 [37] and it has subsequently been adapting to the laboratory environment at a population size of more than 150–200 individuals per generation for ~110 generations (T. Miyatake, personal communication). The population was kept in our laboratory for another ~20 generations prior to the start of the experiments described below. All beetles in the experiments were kept in environmental test chambers at 25°C, 50% RH, and at a 14:10 hr light:dark photoperiod cycle. Our stock population was kept in 2 liter glass jars covered with filter paper at a population size of approximately 500 adult beetles on 200 g of Adzuki beans. Briefly, mating in Adzuki seed beetles is initiated by males who approach females, typically from behind. After a brief antennation of the dorsal surface of females, males then attempt to achieve a genital grasp by protruding their genitalia toward the genital opening of females. Mated females invariably resist copulatory attempts and thwart courting males by kicking vigorously toward them with their hind legs and/or by walking away from males. Males are, however, quite persistent and typically harass females by making repeated copulatory attempts. As in many other taxa, female resistance or avoidance tends to generate nonrandom mating among males in seed beetles [38, 39].

Female Choice Treatments

We varied female choice by experimentally manipulating the efficiency with which females resist male mating attempts. Our experiment includes five different treatment groups (see Supplemental Data), of which three are control treatments. (1) To elevate female resistance efficiency, we provided females with short prongs protruding from their rear abdominal plate. To achieve this, we anesthetized newly hatched virgin females under light CO₂ flow on an open air porous plate and attached clear-plastic prongs (length 2.8 mm, made of 0.2 mm ϕ monofilament fishing line) with glue. The beetles were left under light CO₂ for 40 min to allow for the glue to harden and were then allowed to recuperate for 24 hr prior to experiments. Prongs were attached in such a way that they protruded backward from the female abdominal tip, thus making it more difficult for males to achieve the genital grasp that is required for copulation. (2) As a control for the procedure used to elevate female resistance efficiency, we repeated the manipulation described above with one exception: prongs were instead glued to the elytra such that they did not interfere with male ability to achieve genital contact with females. (3) To reduce female resistance efficiency, we decreased female ability to thwart harassing males with their hind legs. To achieve this, we ablated female hind legs at the midpoint of the tibia. Hind leg ablation to reduce female resistance has been used previously in seed beetles [40, 41]. Leg ablation was performed with microscissors under a dissecting microscope while females were anesthetized with CO₂ (as in

treatments 1 and 2 above). (4) As a control for the ablation procedure used to reduce female resistance efficiency, we repeated the manipulation described above with one exception: instead of ablating hind legs, we ablated one front and one middle leg of each female. (5) As a global control for the experimental procedure, females were only handled under CO₂ anesthesia. Beetles from all five treatments were kept for 24 hr prior to the start of the experiments. To validate that our phenotypic manipulations had the intended effects on female choice, we performed two separate experimental assays (see [Supplemental Data](#)).

Supplemental Data

Supplemental Data include Supplemental Results, Supplemental Experimental Procedures, one figure, and one movie and are available at [http://www.cell.com/current-biology/supplemental/S0960-9822\(09\)01703-5](http://www.cell.com/current-biology/supplemental/S0960-9822(09)01703-5).

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