The reliability of environmental cues shape learning and selection against deleterious alleles in seed beetles

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Abstract

Behavioural plasticity can play a key role in evolution by either facilitating or impeding genetic adaptation. The latter occurs when behaviours mitigate selection pressures that otherwise would target associated traits. Therefore, environments that facilitate adaptive behavioural plasticity could relax the strength of natural selection, but experimental evidence for this prediction remains scarce. Here, we first demonstrate that maternal care in the beetle *Callosobruchus maculatus* is dependent on reliable environmental cues that allow females to reduce larval competition via learning and informed oviposition choices. We show that this facilitation of maternal care relaxes selection against deleterious alleles in offspring. We further find that mothers of low genetic quality generally provide poorer care. However, when receiving reliable environmental cues, the increased opportunity for learning reduced genetic differences in maternal care, further relaxing selection against deleterious alleles. We use our data to illustrate how the identified link between learning of maternal care and the strength of natural selection can impact indirect genetic effects between mothers and offspring and the accumulation of cryptic genetic loads in populations inhabiting environments that differ in their predictability.

Introduction

Phenotypic plasticity plays a fundamental role in shaping organismal responses to changing environments (1–4). Plasticity can help organism to survive and reproduce despite initial adversities. This can allow populations to persist in novel environments and respond to selection, leading to increased genetic change over time (1,2,5,6). However, by mitigating selection pressures, phenotypic plasticity can also reduce the necessity for genetic adaptation (2,7,8). This forgiving nature of plasticity should relax purifying selection and lead to the accumulation of genetic variation that would be deleterious in the absence of plasticity (9–11). If this process continues over time, a population could become dependent on plasticity to survive, as without it, the built up load of conditionally deleterious alleles would become exposed to selection (10,12–14).

The evolution of adaptive plasticity is contingent on the organism's ability to change phenotype in accord with changes in the environment (9,15,16). This leads to the expectation that environments that provide reliable cues of future change should favour the evolution of adaptive plasticity, whereas unpredictable environments may hinder it when environmental fluctuations are too fast, and/or costs of plasticity are too high, to allow accurate phenotype-environment matching (9,15,17–19). Many behavioural phenotypes represent striking forms of plasticity, reflecting an organisms use and processing of information through experience and learning to fine-tune its strategies to fit the perceived environment (3,20,21). Indeed, adaptive behavioural plasticity in the form of learning has been argued to be a key determinant of evolution in novel and fluctuating environments (2,3,9,22,23). Like for other forms of plasticity, however, learning requires reliable environmental cues to result in accurate behavioural adjustments (9,15,24,25). This central role of cue reliability suggests that populations inhabiting environments that differ in their predictability should exhibit systematic differences in learning and genetic load in associated traits. These dynamics are expected to have important consequences for both the levels of segregating genetic variation in natural populations (26,27) and for predicting responses to future environmental change (1–3,6,9,16,28–30).

Here, we investigated how the reliability of environmental information impacts the rate of learning and the resulting strength of selection on deleterious genetic variation in the seed beetle *Callosobruchus maculatus* (**Fig. 1**). We studied behavioural plasticity in maternal care and females' ability to use information to assess future egg-laying opportunities and learn the spatial location of high-quality hosts. The immediate survival benefits of maternal care to offspring are obvious (31,32), but the flip-side of the coin is that by relaxing selection in offspring, maternal care should, at least theoretically, allow deleterious genetic variation to accumulate. Indeed, in burying beetles, populations that are deprived of parental care suffer immediate increases in juvenile mortality and inbreeding depression (33,34), but at the same time seem to more efficiently purge genetic

variation in the long run (11,12,35). Moreover, given that cognitive processes are costly and governed by many genes with pleiotropic effects (9,36–38), the build-up of genetic load via relaxed selection in offspring could feed back on parents' ability to learn and provide care. Genetic variation in parental care is well-known to impact the expression of genetic variation and selection on traits in offspring via indirect genetic effects (*IGEs*: the effect of genetic variation expressed in one individual on trait expression in interacting individuals (26,27,39)). Imagine that parents of high genetic quality provide better care for their young, who also have intrinsically higher survival due to their superior genetic quality. This positive covariance between parent and offspring breeding values increases genetic variance in offspring survival and is predicted to speed up responses to selection compared to a situation where IGEs are absent (26,39,40). Thus, if the reliability of environmental information affects the amount of expressed covariance between adult and juvenile traits, it could have strong effects on evolutionary dynamics (14,28,41).

Female seed beetles provide care for their offspring by preferentially placing eggs on uninfested host seeds, a behaviour that can be finetuned by experience (42–45). First, we gave females the opportunity to choose between uninfested and infested hosts in a spatially complex environment that they were exposed to multiple times. Before and in-between exposures, they received either reliable or unreliable information about the environment's host infestation rate. We show that learning is faster, and care behaviour more accurate, if females receive reliable information about potential future hosts. Second, we find that viability selection in offspring is significantly reduced, and juvenile survival increased, by faster rates of learning and improved care in mothers receiving reliable cues. Third, by comparing the accuracy of care behaviour in mothers of different genetic quality, we find that the improved learning facilitated by reliable environmental information also reduces selection on deleterious alleles in adults. Together, this rendered a 30% reduction in the strength of natural selection and substantially weakened IGEs between maternal care and offspring survival in the treatment providing reliable environmental information. Finally, we adopt an existing theoretical model and parametrize it with our experimental data to illustrate potential population-level consequences of the evolutionary feedback between learning, the strength of natural selection, and the accumulation of conditionally deleterious genetic variation. The results imply that populations evolving exclusively in predictable environments can build up substantial cryptic genetic loads that would make them inferior competitors in unpredictable environments.

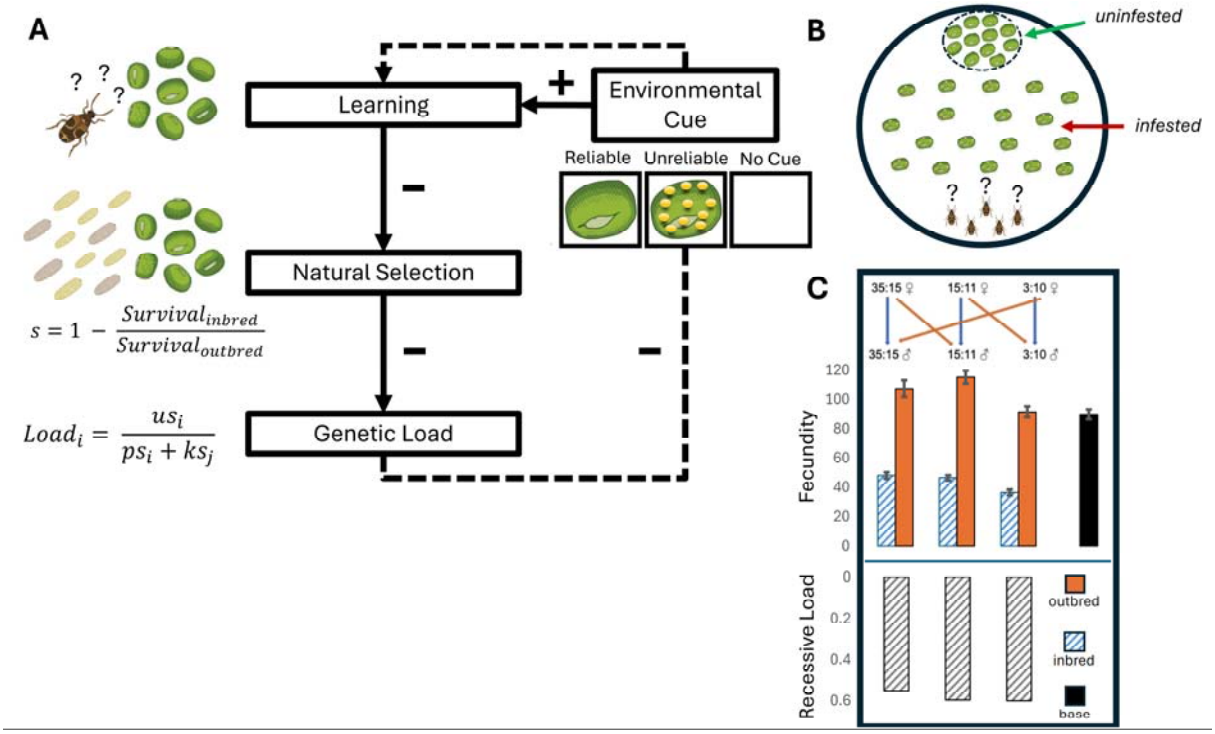

Figure 1: Study design.

A) Hypothesized relationships between the reliability of environmental cues, learning, natural selection, and genetic load. Reliable environmental information is expected to favour adaptive behavioural plasticity in form of learning. Behavioural plasticity can mitigate genetic differences in trait expression and relax the strength of natural selection, thereby increasing the build-up of deleterious genetic variation over time (i.e. genetic load). The accumulation of genetic load could in turn impair learning (broken arrow). However, the degree to which could depend on the available environmental cues used in the learning response. We assessed how environmental cues affected learning of maternal care, and how this in turn affected selection in larval offspring. **B)** Female *C. maculatus* were challenged to find and discriminate among hosts seeds in a spatial learning task. The seeds were either uninfested or infested with eggs of the competitor *C. phaseoli.* We gave females three different treatments before they performed the learning task; a reliable cue giving accurate information about future hosts, an unreliable cue giving false information, and no cue at all. We measured the fitness consequence of maternal care behaviour and learning in each treatment by combining data on female oviposition choice with estimates of larval survival. **C)** Three highly inbred strains (blue stripes) were used and compared to themselves when outcrossed (orange) (base stock population; black). Fecundity estimates per line are given as mean ± 2se. The inbred strains show a recessive genetic load amounting to a reduction in fecundity of 55-60% relative to when outcrossed (grey striped bars). The comparison between the three strains in inbred and outbred state was used to assess the relative strength of viability selection in larvae (*s*) and the effect of recessive genetic load on female learning and maternal care, across the three treatments. Based on these estimates, we predicted the genetic load at mutation-selection balance in a meta-population where individuals received reliable information, *i*, or no information, *j*, at fraction *p* and *k* as a function of the deleterious mutation rate, *u*, and the strength of selection, *s*. See main text for further details.

Methods

Study species

Callosobruchus maculatus is a capital breeding beetle and a pest of leguminous crops common in tropical and subtropical regions. Adults do not require food or water to reproduce at high rates (46,47). Adults typically die 7-14 days after emergence in the absence of food and water in laboratory conditions of 29°C and 50%RH (48). Both sexes can start reproducing on the day of adult eclosion and females lay 70-90% of their eggs during the first few days of reproduction (46). The juvenile phase is completed in 3-4 weeks, and egg-to adult-survival rate on preferred hosts *Vigna unguiculata* (black eyed bean) and *V. radiata* (mung bean) is typically around 90% in laboratory conditions (49–51). Female host search and discrimination is complex. *C. maculatus* uses a wide repertoire of fabaceus host plants (52) but discriminate between high and low quality species (53,54). Females also heavily discriminate against egg-laden seeds and actively avoid competition with other egg-laying females (42,44), as high larval density reduces survival and size at maturity, and thus comes at substantial fitness costs (54–56). *C. maculatus* utilizes both natural habitats where host plant patches are widely distributed, as well as grain storages where adult food is absent but egg laying substrate is abundant and population density is higher (52,54,55). Thus, selection on spatial learning and chemosensory cues is likely strong in natural populations but may take different forms, which is predicted to maintain genetic variation in the traits under study, as seen for other characters related to these alternative environments (57).

Isogenic lines

The three genetic lines used in the experiments were derived from a population isolated from *Vigna unguiculata* (black-eyed bean) seed pods collected at a small-scale agricultural field close to Lomé, Togo (06°10'N 01°13'E) in October and November, 2010 (58). This population harbors abundant genetic variation for life-history traits and fitness correlates (59). Several lineages were exposed to successive full-sib matings (60), and the three most productive lineages used in this study were continued for 30 generations, resulting in near isogenic strains. Thus, the comparisons of these lines in inbred and outcrossed state do not correspond to the effect of natural levels of inbreeding. Rather, the near complete homozygosity prevents further evolution during the experiments and limits within-line variation due to segregation. These lines should therefore be seen as a powerful tool to inspect the fitness consequences of genome-wide homozygosity and estimate the strength of selection across genomic sites harboring recessive deleterious alleles.

To provide an estimate of the inbreeding load in the three lines, we measured fecundity (number of hatched eggs produced over the first week of reproduction) in females that were either produced by within- (inbred) or between- (outbred) line-crosses. Outcrossing between the three lines was performed in a round-robin fashion following a particular schedule (**Fig. 1C**) and should hide the

fitness effects of segregating recessive deleterious alleles, given that these are relatively rare and unlikely to be shared among the three lines. To ensure that bad sperm from inbred males played no part in the fecundity estimates, all females were mated with males from the outbred Lome stock population. We assayed 15 females from each of the six crosses (three inbred and three outcrossed). For comparison we also scored the fecundity of 15 females from the Lome stock population. Each female was placed into a 35mm petri dish filled with mung beans. These petri dishes were placed in a climate cabinet set at standard conditions and the females were left to lay for the next 7 days after which females were removed. After another 7 days beans were removed, and the number of hatched eggs were counted. This showed that inbreeding has led to a decrease in female fecundity of 55-60% relative to the outbred state (**Fig. 1C,** Supplementary 1).

Larval competition

To estimate the fitness effects of variation in maternal care of *C. maculatus*, we let females choose between uninfested mung beans and mung beans infested with larvae of the closely related competitor, *C. phaseoli*, which has strongly overlapping geographical distribution and host plant preference with *C. maculatus* (61–63). The two species also have similar life-histories (64), although the lab stock of *C. phaseoli* has a 20-30% smaller body size than *C. maculatus* (65). The relative success during interspecific competition inside host seeds could be quantified by counts of offspring emerging from beans as the species differ in colour patterning. All experiments were performed using mung beans, which is a preferred host of both species but small enough to typically allow for only one or two adults to emerge (**Fig. 1A**).

Learning and maternal care with and without reliable environmental cues

Based on the previous findings (45) and pilot data, we constructed arenas from 150 mm diameter petri dishes. Each arena consisted of a ‘starting zone’ at one side of the petri dish, where the females were later to be released. Thirty-five mung beans, each infested with 2-4 *C. phaseoli* eggs, were spread out in the area outside of the starting zone. Approximately 35 uninfested beans were placed in a small designated circular area (35mm diameter) opposite to the starting zone (**Fig. 1B**).

Virgin females between 2 and 4 days old were mated to males from the same inbred line. Once mating was over, females were moved individually to a small 35mm petri dish and left there for at least 24 hours without beans (29°C and 12:12LD). Based on a previous study (45), we decided to assay females in groups of five, as this stimulates locomotor activity. A trial started by dividing fifteen mated females equally between three 35mm petri dishes (hereafter known as a ‘holding dishes’). Arenas with their associated holding dishes were placed on a single heating plate set to 30°C. Females spent five minutes in their holding dish, and approximately one minute before being placed in the arena, an environmental cue was added. To one holding dish, five non-infested

seeds were added (reliable cue). To another dish, five highly infested seeds, each with more than 10 eggs, were added (unreliable cue). To the final dish nothing was added (no cue) (**Fig. 1A**). After the final minute with the environmental cue, the females were removed from their holding dish and placed together in the starting zone of the arena and left for 10 minutes during which their locations (on uninfested beans or not) were recorded every 30 seconds. At the end of the 10 minutes, the females were placed back into their holding dish for five minutes and the procedure to introduce the environmental cue was repeated. During this time, the uninfested beans in the arena were replaced. The females were exposed to the arena five times (from hereon: “runs”). We predicted that the reliable cue would make females perform more accurate host choices as these females had gotten correct information about the presence of uninfested seeds. For the no cue and unreliable cue, we expected females to take longer to find the uninfested seeds. For females receiving no cue, we predicted that these females would be less stimulated to start their search for hosts, as they yet had received any information about the presence of hosts, and that they might regard the hosts with competitor eggs acceptable in the absence of other obvious alternatives. For females receiving the unreliable cue, we predicted that these females would spend more time inspecting the infested seeds found in the arena and be more willing to lay eggs on them, as these seeds are of comparatively good quality relative to the seeds that the females had been exposed to in the holding dish before each run.

The first 12 trials were conducted with two environmental treatments simultaneously (6 with reliable versus unreliable cue, and 6 with reliable versus no cue). Once the observer was used to the protocol, we included all three environmental treatments simultaneously, to run an additional 24 trials. The observations were conducted without blinding the observer due to practical limitations. However, to assess the potential for observer-bias, we later repeated the behavioral assays with a blind observer, conducting another 12 trials using the Lome base population. This showed that all three experiments produced qualitatively similar result (summarized in Supplementary 1). We therefore combined all data for the final analysis presented in the main text, based on 66,000 observations of behavior from 660 females in total.

Learning was defined as the improvement between runs (from 1-5) in the rate that females found the patch with uninfested seeds. Thus, to evaluate the effect of environmental cues on learning, we first analyzed the number of females found on uninfested beans in each arena as a binomial response using mixed effects generalized linear models using the lme4 package (66) for R (67). We included fixed effects of time (10 minutes within each run) and run (1-5) crossed with treatment (reliable/unreliable/no cue). Time and run were both added as continuous variables fitted with a second-degree polynomial. We included female age and the line ID as additional fixed effects. We included the trial ID as a random effect crossed with treatment. We inspected the consistency of the responses in the three lines with reference to treatment and inbreeding status. This showed

that the three lines behaved qualitatively the same, and we report a summary of line comparisons in Supplementary 1.

We also quantified differences in learning between the three environments by comparing the number of eggs laid on infested and uninfested seeds from the two non-blinded experiments for a total of 480 females scored in fives over 96 trials. One model was run with a binomial response variable to inspect the fraction of eggs laid on the two types of seeds at the end of each trial. This model included environmental cue and line ID as fixed effects. The second model analyzed the number of eggs laid on the uninfested (high-quality) hosts during each of the five consecutive runs as a Poisson distributed response variable. This model included environmental cue, run, and their interaction as fixed effects, and trial as a random term.

Maternal care and the strength of selection on recessive deleterious alleles

To estimate the fitness consequences of variation in maternal care, we tracked survival of *C. maculatus* larvae competing with *C. phaseoli*. To estimate the strength of selection on recessive deleterious alleles at different levels of competition, we compared survival of inbred and outbred *C. maculatus* larvae. We achieved this by mating parents either within (inbred) or between (outbred) lines (**Fig .1C**), reasoning that survival differences between inbred and outbred larvae should reflect natural selection against recessive deleterious alleles revealed by homozygosity in inbred individuals (68). The experiment was carried out in two temporal blocks. Two females (mated either within or between lines), were placed in a 35mm petri dish with mung beans. The seeds were either uninfested or infested with a known number (1, 2, 3 or 4) of *C. phaseoli* eggs that had been laid on the seeds a week earlier (as in the behavioural trials). After 72 hours, the females were removed from the petri dishes and the beans were taken out and checked for eggs. If new eggs were found, they were counted, and beans were placed individually into 24-welled chambers. These chambers were placed into climate cabinets set at standard conditions and were later checked to see how many adults that had emerged of each species.

The data consisted of three replicates (each of two females) per line, inbreeding status, and competition level (0-4 *C. phaseoli* eggs), for a total of 60 female duos, laying 8323 eggs on 2450 mung beans. We analysed the survival of *C. maculatus* as a binomially distributed response, with inbreeding status (inbred/outbred), the competition intensity, and their interaction as fixed effects. The ID of the female duo was added as a random effect. Note that we chose to sum the eggs of both *C. maculatus* and *C. phaseoli* as a measure of competition intensity, as mung beans had 1-3 *C. maculatus* egg laid on them, with one egg being most common and three eggs rare. Seven seeds had more than three *C. maculatus* eggs on them. We chose to discard these from the

analysis since they were unevenly distributed with six of seven seeds from outbred females. A summary of the distribution of eggs laid by both species is presented in Supplementary 7.

To predict the strength of selection, we refitted the mixed effect model within a Bayesian framework using the MCMCglmm package (69) for R. Based on the posterior estimates of model coefficients transformed from log-odds back to original scale, we calculated the strength of selection (with 95% credible intervals) for different levels of competition, *c*, as : $\Delta\omega_c = 1 - \frac{v_{c,I}}{(v_{c,O})}$, where v_I and v_O is viability of inbred and outbred offspring, respectively. We ran models for 100,000 iterations, preceded by 50,000 burn-in iterations, and stored every 100th iteration, resulting in 1000 uncorrelated posterior estimates of model coefficients. We used flat and weak priors for the random effects.

Learning and maternal care by mothers of different genetic quality

Using the same set-up as for the original behavioural assays, we quantified the effect of inbreeding load on females' ability to use the environmental cues and adjust their maternal care behaviour. For this we compared females from within (inbred) and between (outbred) line crosses. To minimize the risk that inbred males' sperm would negatively affect female fecundity and their motivation for host search, all females were mated with outbred males from the Lome stock population. In each trial, the three cue treatments were included. Nine trials were run with inbred females and nine with outbred females (i.e. three replicates of five females per line and cross type). This resulted in a total of 27,000 behavioural observations from 270 females. The data was analysed by the same model fitting procedure as for the first experimental data with the addition of adding inbreeding status (inbred/outbred) as a fully crossed fixed effect.

Genetic load in populations receiving reliable and unreliable environmental information

To quantify the fitness cost associated with the revealed recessive deleterious alleles in each of the three cue treatments, we refitted the mixed models on juvenile survival (see Fig. 4) and female behavioural plasticity (see Fig. 6) in inbred and outbred individuals using a Bayesian framework. This allowed us to calculate the predicted strength of selection against recessive deleterious alleles based on model posterior distributions. Fitness (ω) of inbred and outbred genotypes was calculated by weighting the number of eggs laid on infested and uninfested seeds by the predictions for larval survival at no competition (uninfested seeds) and three competitors (the mean number of *C. phaseoli* eggs on infested seeds in the behavioural experiments). The genome-wide strength of selection against deleterious alleles in treatment *j* was then calculated as: $s_j = 1 - \omega_{i,j} / \omega_{o,j}$. Using the estimates of *s* we then predicted the long-term fitness consequences and genetic load at mutation-selection balance in environments corresponding to the reliable cue and no cue

treatment of our experiment using a previously published model by Kawecki et al. (1997) **(13)**. Details are described in Supplementary 6.

Results

Learning and maternal care with and without reliable environmental cues

We first explored whether the reliability of environmental cues affected females' ability to learn and express maternal care. Within each of the five runs of a trial, females explored the arena, resulting in more females finding the patch with uninfested seeds as time progressed (χ^2_2 = 4851.9, p < 0.001, Fig. 2). There were clear signs of learning during the trials, signified by a general increase in the proportion of females finding the uninfested hosts between the five consecutive runs (χ^2_2 = 5818.0, p < 0.001, Fig. 2). There was evidence for increased learning in females receiving reliable cues compared to those receiving an unreliable cue or no cue (Fig. 2). This was evident as an effect of treatment on the proportion of females finding the uninfested hosts (χ^2_2 = 108.8, p < 0.001) and as a difference between treatments in how this proportion increased between runs (Treatment:Run interaction: χ^2_4 = 72.9, p < 0.001, Fig. 2). Because females receiving the reliable cue found the uninfested hosts faster compared to females in the two other treatments, there was also a stronger saturation in the proportion of females found on the uninfested hosts with time in the reliable cue treatment (Treatment:Run:Time interaction: χ^2_4 = 116.7, p < 0.001, Fig. 2). Summary of results in Supplementary Table 2.

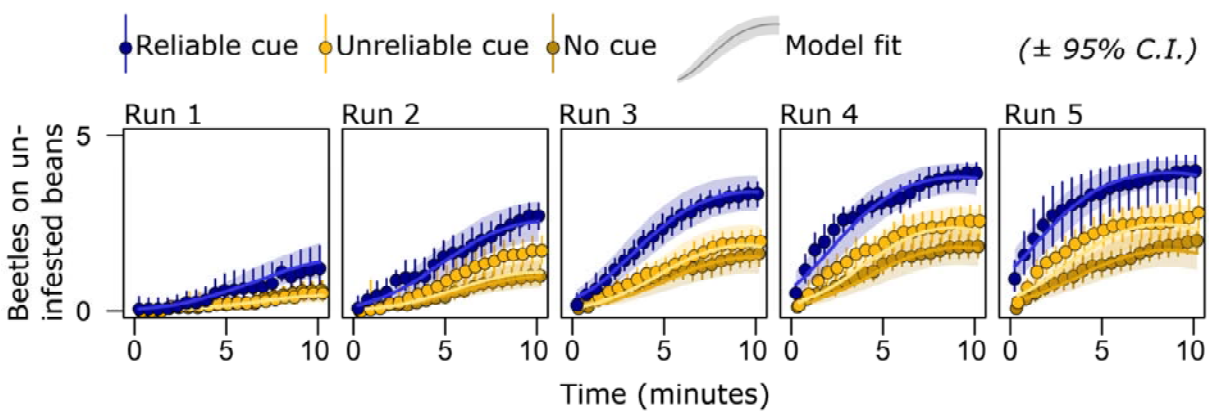

Figure 2: The effect of environmental information on female host search
With time, more females located the patch with uninfested host seeds both within and between runs. The improvement over runs demonstrates learning during the experiment. The rate of learning was highest in females receiving the reliable cue (blue) compared to those exposed to the unreliable cue (yellow) or no cue (brown).

Females receiving the reliable cue also laid a larger fraction of eggs (χ^2_2 = 204.3, p < 0.001, Fig. 3, Supplementary Table 3A), and more eggs in total (χ^2_2 = 224.4, p < 0.001, Fig. 3, Supplementary

Table 3B), on uninfested seeds. Thus, reliable environmental cues, providing the opportunity for learning of maternal care, are likely to reduce competition in larval offspring.

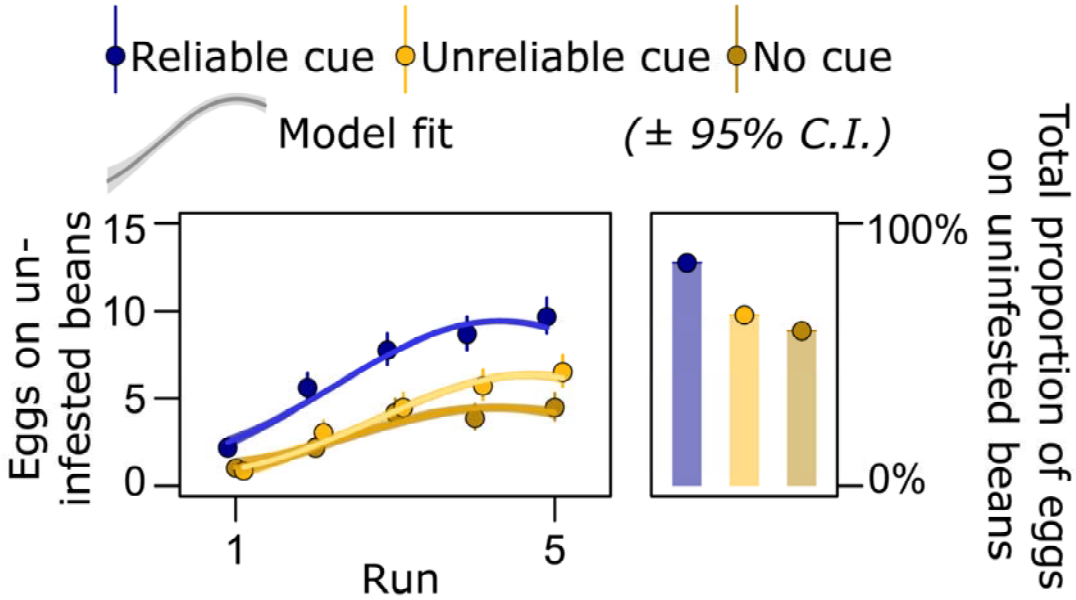

Figure 3: The effect of environmental information on female host choice
Females laid more eggs on uninfested hosts in later runs of the experiment, showing that they got better at locating the patch with uninfested seeds. The rate of improvement was highest in females exposed to the reliable cue (blue) compared to those exposed to the unreliable cue (yellow) or no cue (brown). This also resulted in females receiving the reliable cue placing a higher proportion of their eggs on uninfested seeds relative to females in the other treatments.

Maternal care and the strength of selection on recessive deleterious alleles

To estimate the fitness consequences of variation in maternal care, we tracked survival of *C. maculatus* larvae competing with its close relative, *C. phaseoli*. As expected, the density of competitor eggs had a negative impact on egg-to-adult survival of *C. maculatus* (χ_1^2 = 52.6, p < 0.001, Fig 4). To estimate the strength of selection on recessive deleterious alleles expressed in offspring, we compared the effect of larval competition in inbred and outbred *C. maculatus larvae*. As expected, inbreeding reduced larval survival (χ_1^2 = 49.2, p < 0.001, Fig. 4). Moreover, inbred larvae suffered more from the effect of competition compared to outbred offspring (Inbreeding:Infestation intensity interaction: χ_1^2 = 3.72, p = 0.035, Fig. 4, Supplementary Table 4), confirming that competition increases selection on genetic quality.

Interaction coefficients in logistic regression models represent deviations from multiplicative effects on odds ratios. However, the relevant currency in the eyes of natural selection is given by relative differences in survival among genotypes. Therefore, we estimated the strength of natural selection against recessive deleterious alleles by comparing the relative survival of outbred and inbred larvae at low density (no competition) and high density (six competitors – the maximum observed

within our data range) based on estimates from a Bayesian mixed effect model, with the strength of selection at the level of competition, *c*, proportional to: $\Delta\omega_c = 1- \frac{\quad}{\quad}$, where v_I and v_O is viability of inbred and outbred larvae, respectively. This yielded a fitness cost of deleterious recessives equal to: $\Delta\omega_{C=0} = 0.20$ (95% CI: 0.05-0.37) with no competition, and: $\Delta\omega_{C=6} = 0.84$ (0.67-0.94) at high competition, implying a roughly four times greater strength of purifying selection.

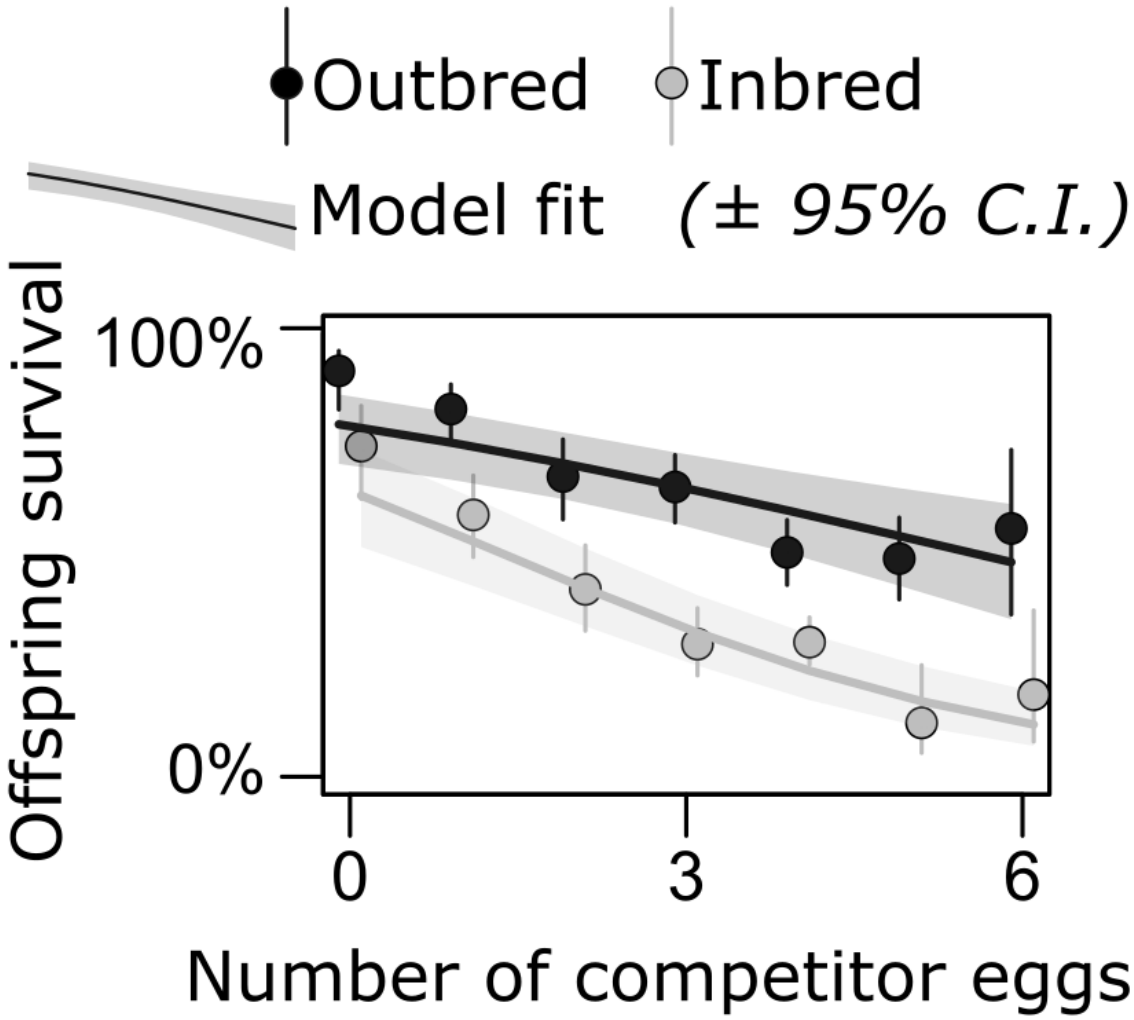

Figure 4: The fitness consequences of larval competition
Eggs placed on seeds with more competitor eggs suffered lower survival. This effect was stronger in inbred (grey) compared to outbred (black) larvae, resulting in stronger selection against deleterious alleles at high competition.

Learning and maternal care by mothers of different genetic quality

We compared the ability of inbred and outcrossed females to assess the environmental cue and improve maternal care by learning. A larger proportion of the females receiving the reliable cue

were found in the patch with uninfested seeds compared to females in the two other treatments (χ^2_2 = 177.2, p < 0.001), replicating the results seen in the previous experiment (compare Fig. 2 and Fig. 5). Outcrossed females performed better care compared to inbred females (χ^2_1 = 16.6, p < 0.001). Interestingly, however, the difference diminished with time both within (χ^2_2 = 117.8, p < 0.001) and between (χ^2_2 = 223.0, p < 0.001) runs, suggesting that inbred females took longer to find the patch with uninfested seeds, but then performed like outcrossed females in the last run of each trial (Fig. 5). This indicates that learning can diminish innate genetic differences in maternal care. Importantly, this ameliorating effect of learning was strongest in females receiving the reliable cue (Treatment:Inbreeding interaction: χ^2_2 = 14.6, p < 0.001; Treatment:Inbreeding:Run interaction: χ^2_4 = 24.3, p < 0.001, Fig. 5, Supplementary Table 5).

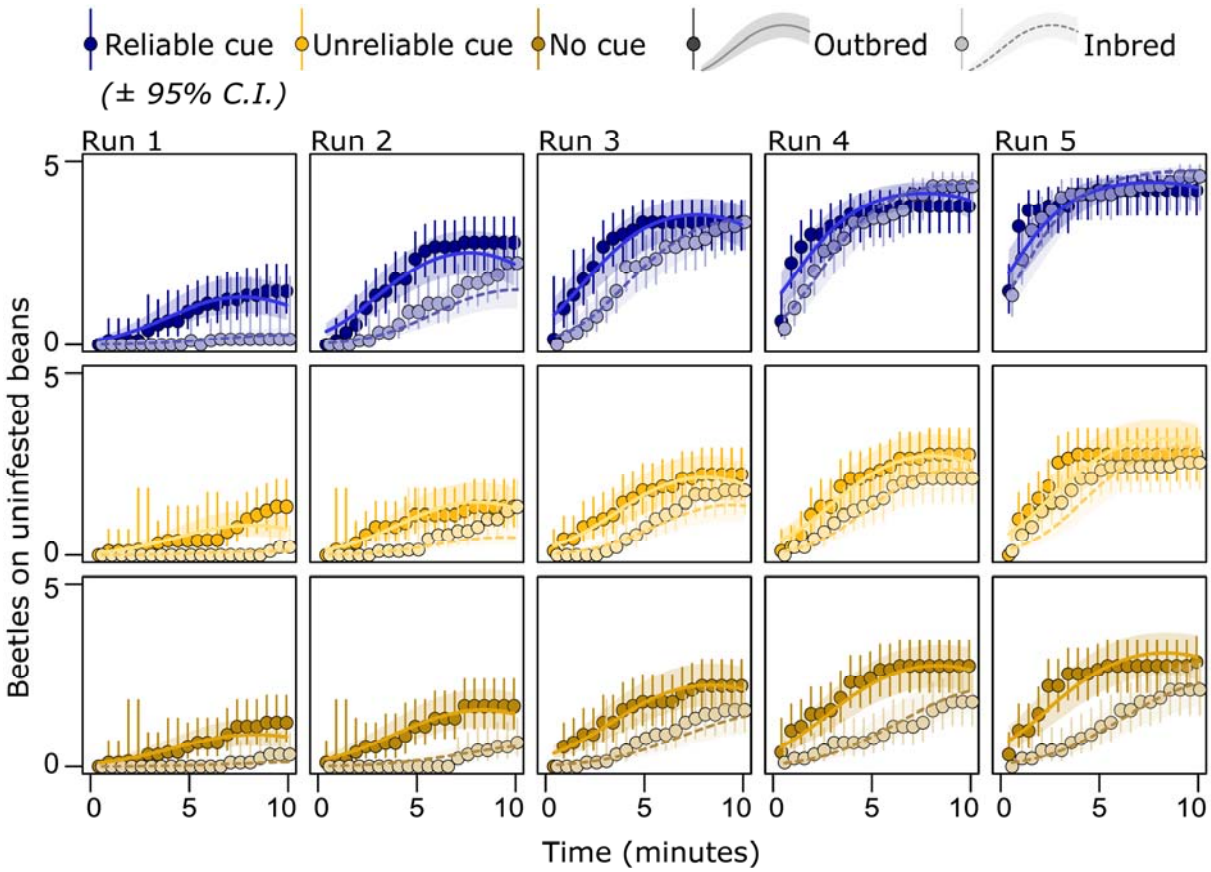

Figure 5: Host search in females of different genetic quality
Females learnt the location of the patch with uninfested host seeds as runs progressed. The rate of learning was again highest in females exposed to the reliable cue (blue) compared to those exposed to the unreliable cue (yellow) or no cue (brown). Inbred females were poorer at finding uninfested seeds. However, learning reduced this difference between outbred and inbred females, especially if females received the reliable cue.

When inspecting the number of eggs laid on host seeds, we found that females receiving the reliable cue laid both a larger fraction of eggs (χ^2_2 = 72.8, p < 0.001) and overall more eggs (χ^2_2 =

98.3, p < 0.001) on uninfested seeds compared to females in the two other treatments, again replicating the results seen in the previous experiment (compare Fig. 3 and Fig. 6). Outcrossed females performed better compared to inbred females, both with regards to the fraction (χ_1^2 = 13.4, p < 0.001) and total number (χ_1^2 = 33.2, p < 0.001) of eggs laid on uninfested seeds. For the fraction of eggs on uninfested seeds, we found no effect of the environmental cue on the difference between inbred and outcrossed females (Treatment:Inbreeding interaction: χ_2^2 = 3.88, p = 0.14). However, for the total number of eggs on uninfested seeds, the difference between inbred and outcrossed females was smaller in the reliable cue treatment (Treatment:Inbreeding interaction: χ_2^2 = 11.7, p = 0.003, Fig. 6). This result lends further supports to the hypothesis that reliable environmental cues that leave opportunity for learning can ameliorate individual fitness differences in maternal care owing to genetic quality.

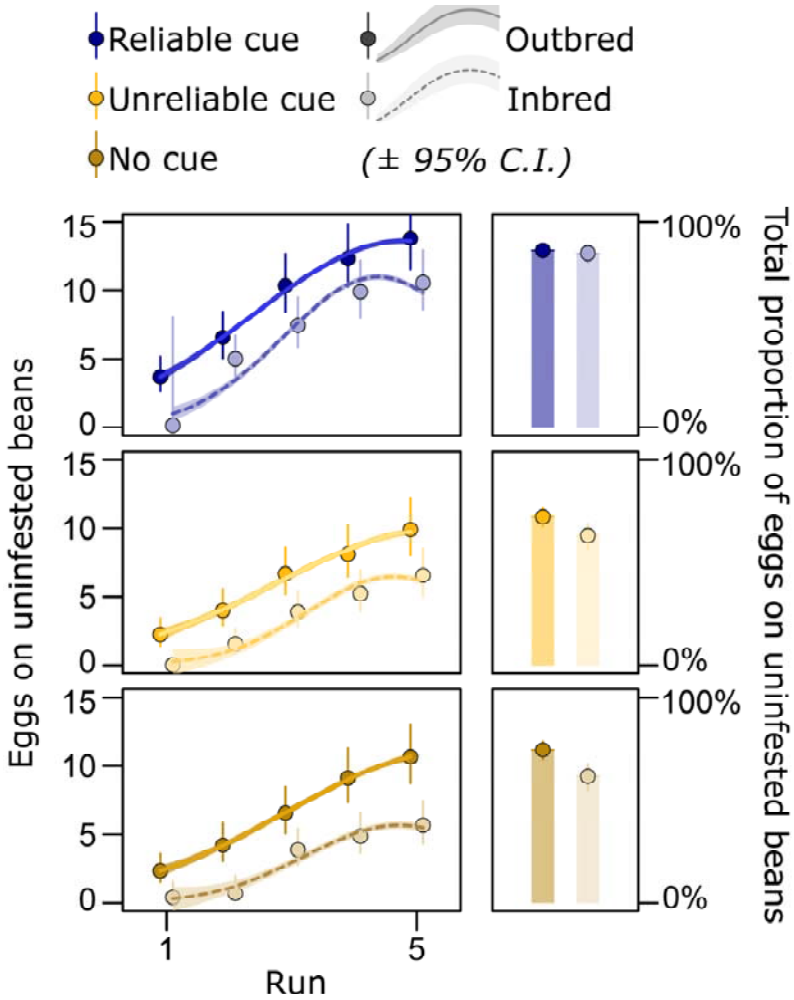

Figure 6: Host choice in females of different genetic quality
Females got better at locating the patch with uninfested seeds as runs progressed. The rate of improvement was highest in females exposed to the reliable cue (blue). Inbred females laid fewer eggs on the uninfested seeds. However, when receiving the reliable cue, inbred and outbred

females showed smaller relative differences in the number and proportion of eggs placed on uninfested seeds compared to when receiving the unreliable (yellow) or no (brown) cue.

Combining the information on host choice and juvenile survival across the three genetic lines in inbred and outcrossed state, we estimated the genetic covariance between genotype values for maternal care and offspring survival in each environmental cue treatment. The reliable cue diminished genetic (co)variation between mother and offspring genotype, thus reducing the strength of IGEs relative to the treatments with unreliable cue and no cue (Fig. 7).

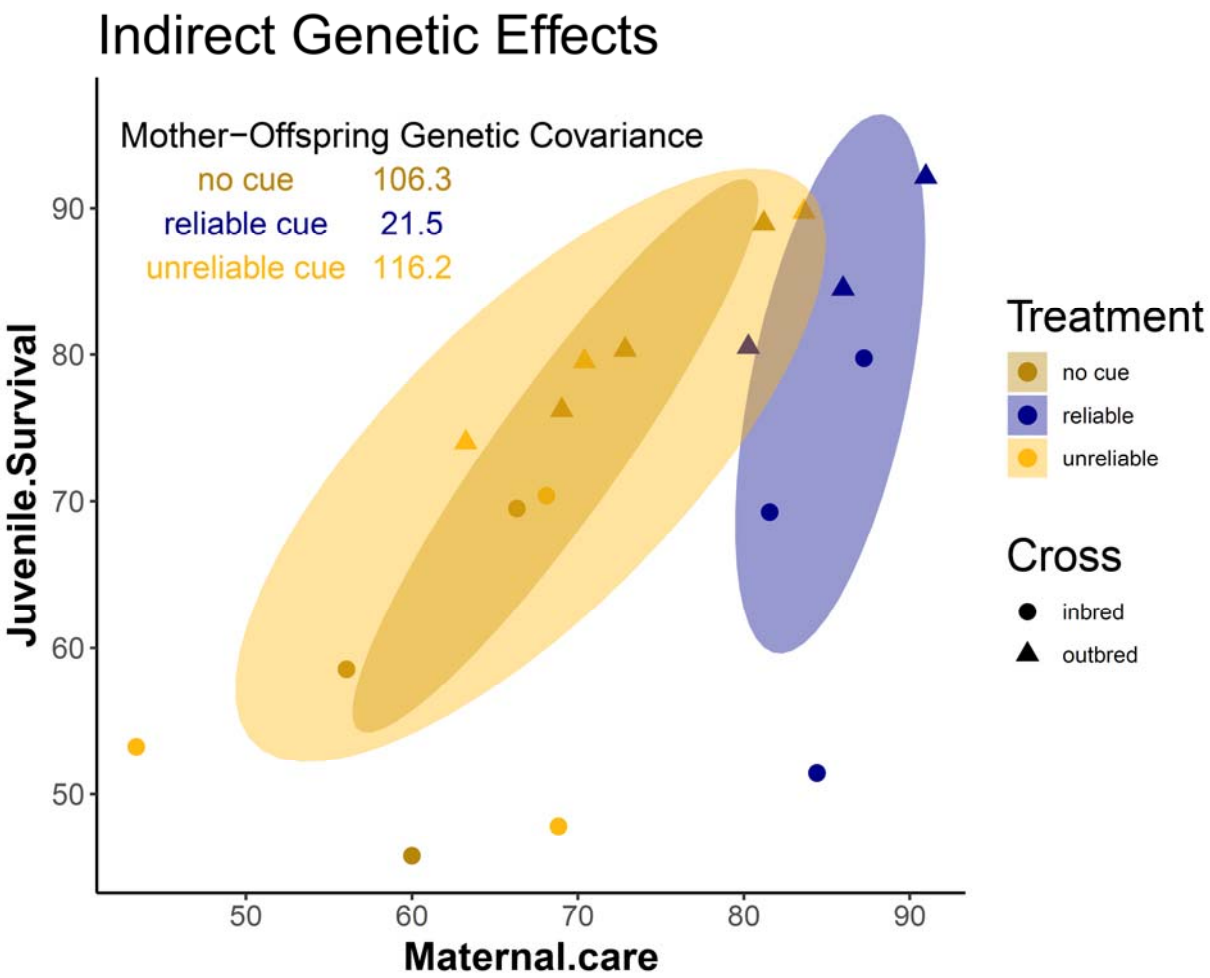

Figure 7: Indirect genetic effects depend on environmental information
Shown are genotype values for maternal care (percent eggs laid on uninfested hosts) and juvenile survival (percent expected survival to adulthood) in the treatment with a reliable cue (blue), unreliable cue (yellow), and no cue (brown). Outcrossed genotypes are shown as triangles and inbred isogenic lines as circles. There is substantially more genetic covariance between mother's care and their offsprings' survival in the treatments with no cue or an unreliable cue relative to the treatment providing reliable information. Shown are 68% confidence ellipses (corresponding to one standard deviation) based on genotype values.

Genetic load in populations receiving reliable and unreliable environmental information

We quantified the relative fitness cost associated with recessive deleterious alleles in each of the three treatments by comparing fitness of inbred and outbred genotypes. Fitness was calculated by weighting the total number of eggs laid on infested and uninfested seeds (Fig. 6) by the predictions for larval survival at no competition (uninfested seeds) and three competitors (the mean number of *C. phaseoli* eggs on infested seeds during behavioural trials) (Fig. 4). Expected fitness was higher in the treatment providing reliable cues compared to the other treatments (Fig. 8A). Moreover, and echoing the previous findings, the reduction in fitness due to recessive deleterious alleles was lower in the treatment with a reliable cue ($\Delta\omega$= 48%, CI: 29-61%) than in the treatment with a unreliable cue ($\Delta\omega$= 60%, CI: 45-70%, P_{MCMC} = 0.062) and no cue ($\Delta\omega$= 64%, CI: 52-74%, P_{MCMC} = 0.006), although the difference was marginally non-significant in the first comparison (Fig. 8B, C).

Hence, in accordance with theory, reliable environmental information, facilitating learning, rendered higher fitness and population productivity, while at the same time relaxing selection against deleterious alleles. We predicted the long-term fitness consequences of learning in environments corresponding to our reliable cue (i.e. a predictable environment) and no cue treatment (a relatively more unpredictable environment), respectively, by parameterizing an existing model by Kawecki et al. (1997) (13) with our treatment-specific estimates of selection and productivity (full details are given in Supplementary 6). This model estimates the genetic load at mutation-selection balance in a meta-population experiencing two different environments that differ in the strength of selection on deleterious alleles. Based on our experimental data, we set the mean strength of selection in the unpredictable environment providing no information to be 0.64/0.48 times stronger than in the predictable environment providing reliable information (i.e. $s_k = 1.33s_j$, Fig. 8B, C). The results how that, while fitness is always greater in the predictable environment with a reliable cue, the frequency of deleterious alleles increases in the population as the environment becomes more predictable due to the alleviating effect of learning on the strength of selection (Fig. 8D). The resulting fitness effects of this accumulated genetic load are more pronounced at high mutation rate and in the unpredictable environment. Thus, in certain scenarios (high reliability of environmental information and high mutation rate), it could be envisioned that species evolving in predictable environments will be unable to colonize unpredictable environments due to competitive exclusion by resident species.

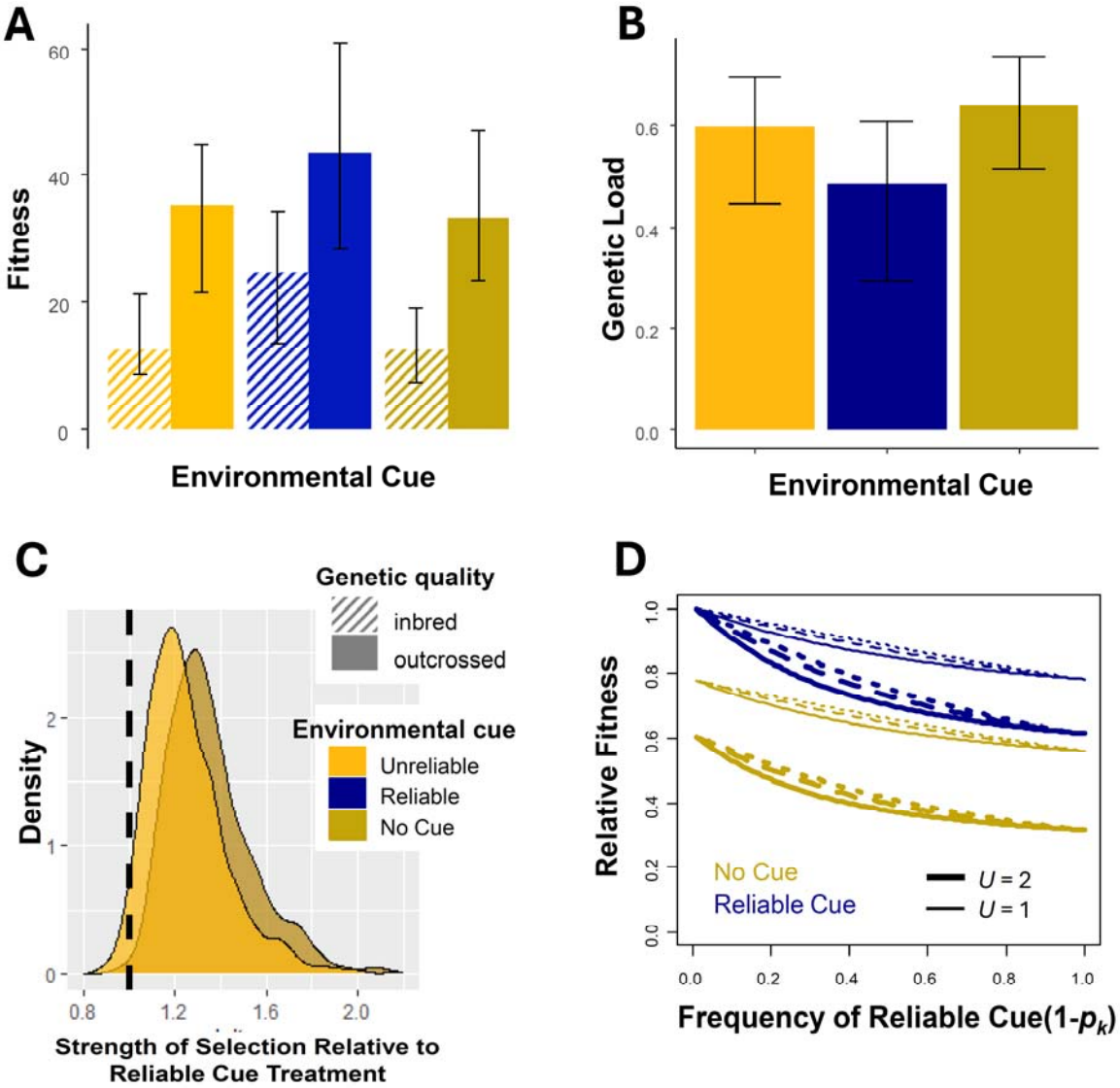

Figure 8: Genetic load in predictable and unpredictable environments.
In A) Expected offspring production is highest in the treatment providing a reliable cue compared to the other two treatments. B) The strength of selection against deleterious recessive alleles in inbred individuals is reduced in the reliable cue treatment relative to the other treatments. C) Bayesian posterior densities showing the increase in the strength of selection in the treatments with unreliable cue and no cue compared to the treatment with a reliable cue. D) Evolution in a predictable environment (corresponding to the reliable cue treatment) is predicted to relax selection on deleterious alleles relative to evolution in an unpredictable environment (corresponding to the no cue treatment), leading to higher fitness but also an accumulation of genetic load in the meta-population. This effect increases for higher deleterious mutation rates (thick lines: U = 2, thin lines U = 1, set to capture the range of probable values in eukaryotes (70)). Load increases further when the predictable environment is more productive (full lines: B_i/B_k = 1.96 based on the difference in expected fitness of inbred individuals in the treatment with a reliable cue relative to those in the treatment with no cue, hatched lines: B_i/B_k = 1.30 based on the same difference between outbred individuals, and dotted lines: $B_i = B_k$ for comparison). Given that the deleterious mutation rate is not negligible, evolution with learning in the predictable environment will increase load in the unpredictable environment substantially. Further details are given in Supplementary 6.

Discussion

Finetuning of behaviours can allow organisms to avoid perils and shield them from selection. Behavioural plasticity in the form of learning is therefore regarded as a central mechanism allowing animals to cope with fluctuating environments (2,7,22,26). However, by relaxing the strength of selection overall, behavioural plasticity may lead to the accumulation conditionally deleterious alleles, which' effects may become exposed in the absence of mitigating behaviours. If this process continues for long enough, organisms practising extensive behavioural plasticity are predicted to build up substantial conditionally deleterious genetic loads (9,27,39), perhaps to the extent that they no longer will be competitive in situations where adaptive plasticity is restricted by too rapid and unpredictable environmental changes (70,71). Indeed, to accurately finetune behaviours by learning, the organism needs to be able to assess and predict environmental variability (9,15), leading to the expectation that organisms occupying environments that differ in their predictability may show systematic differences in the expression of learning and conditional genetic loads.

Here, we sought an experimental proof-of-principle for this hypothesis. By manipulating environmental cues given to egg-laying female seed beetles, we show that learning is strongly dependent on reliable information on future host quality and location. Reliable environmental cues greatly improved maternal care, in turn reducing genetic selection in juveniles. This relaxed selection is expected to lead to the accumulation of a cryptic genetic load over time. We show that increased genetic load is likely to feed back on the female's ability to perform maternal care; inbred mothers were significantly worse at finding uninfested hosts. However, inbred females learned the location of uninfested hosts with time, diminishing genetic differences in maternal care. Importantly, the improved learning facilitated by reliable cues contributed further to relaxing selection on genetic load. Hence, our study presents evidence for a feedback loop between environmental predictability, adaptive behavioural plasticity in form of learning, and the strength of purifying selection.

Our study also provides an experimental demonstration of how the strength of indirect genetic effects (IGEs) can be moulded by the environment. We identified effects of recessive deleterious alleles on both the ability of mothers to provide care and on their offsprings' survival, such that (outbred) mothers of high genetic quality provided better care and increased survival of their offspring, who also had higher intrinsic viability. This positive genetic covariance between the mothers trait (care) and the expression of her offsprings' trait (survival) increases genetic variance in offspring fitness relative to a situation without IGEs (26,39,40), and should lead to more efficient purging of genetic load. However, this positive covariance was considerably weakened in the treatment providing reliable cues that reduced genetic differences between both adults and offspring, demonstrating that environmental variation can be a key component of evolutionary dynamics driven by IGEs.

C. maculatus females use the presence of eggs to choose whether or not to oviposit (42). However, even though a mother can use cues to make informed decision on where to oviposit, she cannot protect the offspring from the choices made subsequently by other mothers ovipositing on the same host. Likewise, larval genotypes that compete will constitute an evolving environment that may favour the evolution of even more (or less) pronounced competitive traits. This illustrates that environmental predictability in this system is likely to evolve as a function of genes and the IGEs exerted between both competing females and their juvenile offspring (56). Similar dynamics have been elegantly demonstrated in burying beetles (12,35,72), and as we argue here, the increased opportunity for learning of maternal care may have strong impact on these processes. It is possible that the interplay between environmental variation and IGEs may be a key driver of geographic differentiation in strategies of host use in this system. Indeed, different geographic strains of *C. maculatus* show genetic differentiation in foraging strategies (73) but see: ((74)) and novel host use (50). Host specialization has been shown to evolve rapidly in *C. maculatus* (53,75) and future studies could more directly explore the role of IGEs in driving local adaptation in this system, which may provide important insights into the rapid geographic spread of this insect pest.

While our chosen treatments represent plausible situations for egg-laying *C. maculatus* females, they do not perfectly match environmental variation and frequencies of informed and uninformed oviposition choices in natural populations. Nevertheless, because female *C. maculatus* are faced with ephemeral host availability and varying population densities and levels of competition (42,49,50,50,54,55,57), variation in the predictability in host quality and abundance is likely ubiquitous. Our treatments were chosen to capture this natural variability, but our results should mainly be taken as a proof-of-principle that environmental variation is key in determining the adaptive value of learning, with cascading effects on the strength of selection and genetic load. To illustrate this point further, we analysed effects on juvenile survival of the competitor *C. phaseoli* when coinhabiting seeds with inbred and outbred *C. maculatus* larvae. This showed that also competing species can be affected by the dynamics between environmental variation and learning (Supplementary 7). Indeed, our adoption and parameterization of the model on genetic load in heterogenous environments by Kawecki et al. (1997) (13) illustrates that learning in predictable environments can build up a sizeable conditional genetic load that becomes exposed to selection upon contact with competing species evolving without learning. By comparing an environment giving perfect cue reliability with an environment giving no cue at all, in a situation where heavily infested hosts were presented before high quality hosts (thus skewing the placement of eggs towards infested hosts), we likely overestimated the cost associated with environmental unpredictability. Still, it is worth noting that by assuming homogeneous selection across genomic loci within each environment, and by focusing only on deleterious alleles (i.e. no antagonistic

pleiotropy across environments), our calculations underestimate the magnitude of the (hidden) genetic load built up in the predictable environment (76).

To conclude, we find that beetles efficiently provide maternal care when they can learn from previous experience using reliable environmental information. This, in turn, relaxes selection on offspring, allowing for the accumulation of genetic load. We show that the accumulation of load is likely to impact maternal care itself, but that genetic differences in maternal care can be ameliorated by improved learning in environments providing reliable information. The identified eco-evolutionary feedback between learning and the strength of selection is predicted to substantially weaken IGEs between parental provisioning and offspring survival and lead to greater genetic loads in populations inhabiting predictable environments.

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